A TEXT BOOK OF CYTOLOGY

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(For University Students)

By

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PREFACE TO THE THIRD EDITION

The authors feel amply rewarded to find that this book is getting due popularity and favour from the students and teachers of the country. This prompted the authors to undertake its thorough revision. In this process, the book remained out of market for sometime which the authois regreat very much.

The older volume was very carefully gone through page by page and changes have been made wherever it was thought to be desirable in order to make the subject matter up-to-date, to clarify statements and to correct the errors. Two new chapters on "Cell metabolism" and "Effect of radiation on Bioplasm" have been incorporated. The chapter on "Nucleic acid and nucleic acid synthesis" has been bifurcated into two chapters, entitled as "Nucleic acid and nucleases" and "Nucleic acid synthesis". Besides, several new figures have been added ; several others which lacked clarity in the old edition have been redrawn. Summary has been given at the end of each chapter. Index has also been incorporated in the end In short, authors believe that the present edition has greatly improved and will be more handy and serviceable to the students and teachers.

To all those, who have helped the authors in revising this text, directly or indirectly, they express their gratitudes. In the end the authors desire to express their sincere appreciation and thanks to Mr. K. N. Gupta, Managing Partner, Jai Prakash Nath & Co., Meerut and his entire staff for their constructive and sincere efforts in making the author's publications a success

Comments and suggestions from readers are always welcomed and gratefully acknowledged by the authors.

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June 20, 1970.

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PREFACE OF THE FIRST EDITION

The book *"A Text Book of Cytology"* is intended primarily for undergraduate students. Since the cytology has now become a part of the zoology syllabi of some of the Indian universities, a book of this type was largely felt. In this miniature book, the authors have tried to give comprehensive, to the point and up to-date description of the cellular components. While preparing the book they have kept in mind the difficulties of the average students. The language is kept simple and the diagrams simplified to make them clear and easy to understand.

In writing this book, the authors have freely consulted other standard texts. To all those sources they are thankful. In particular, thanks are due to Mr. H. C. Goel, M. Sc, Asst. Prof. of Zoology, D. A. V. College, Muzaffarnagar; Mr. P. C. Garg, M.Sc., Asst Prof. of Zoology, D. A. V. College, Ambala, and Mrs. Meera Saxena, M. Sc. The help and co operation rendered by Mr. K. N. Gupta, Managing Partner, Jai Prakash Nath & Co., Meerut has been indispensable. This venture owes its success to him.

The authors will gratefully acknowledge the helpful criticism and suggestions of the teachers and the students as this will help them in making the book better in the subsequent editions.

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July 1968

DALELA
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CONTENTS

INTRODUCTION

Cytology (*Cyto*=cell+*logos*=discourse) is a specialised branch of biology which deals with the study of finer structure of the cell, its parts and their physiology, and the mechanism of cell multiplication, heredity and development. Today cytology concerns itself particularly with germ cells but should not and in reality does not exclude from its scope the significant features of cells other than the germinal.

HISTORY OF CYTOLOGY

Discovery of cell—The discovery of the cellular structure of organism is intimately bound up with the invention of the compound microscope (Gr : *Mikros*=small ; *skopein*=to see.) During seventeenth and eighteenth centuries, a number of students of minute anatomy, demonstrated that the tissue of an organism has a cellular organisation. The earliest published picture of such structure was presented by **Robert Hooke** in 1665 before the Royal Society of London. The results of his investigations on the 'texture of cork by means of magnifying lenses' was the point of departure for all knowledge concerning the microscopic organisation of living matters. He described the cork as composed of "small spaces surrounded by firm wall" to which he gave the name "cell" (Gr : *kytos*=hollow space). At that time the term cell meant two things : simple cavities bounded by walls like the cells in a honey comb or globules of numerous unrelated kinds. For long they were regarded as subordinate components of tissue rather than important individualized units. Also in 17th century **Grew** and **Malpighi** repeated the observations of Hooke in different plants and recognized in them minute cavities in the midst of homogeneous mass which they called "utricles" or "vesicles". This knowledge practically remained stationary until the beginning of 19th century.

Cell Theory—The theory that all animal and plant organisms are composed of cells is associated with the names **Schleiden** (1838) and **Schwann** (1839) but prior to them several workers sponsored the same views in a form more or less complete. **Mirbel** (1808-09) suggested that, "plants are formed by a membranous cellular tissue." Similarly **Lamarck** (1819) said, "no body can have life, if its constituent parts are not cellular tissue or are not formed by cellular

tissue." Similar ideas were also put forward by Turpin (1826), Meyen (1830) and von Mohl (1831).

Schleiden, a German botanist and Schwann, a German zoologist, used for the first time the term **Cell theory** by stating: "The cells are organisms; and animals as well as plants are aggregates of these organisms, arranged in accordance with definite laws". Schwann's results established the cell theory in a definite form. Schwann had the clear ideas not only of the morphologic importance of the cell but also on its physiologic significance. According to him cellular phenomena comprises two groups, *i. e.* the plastic phenomena and the metabolic phenomena. The former corresponds to cellular morphology and includes in itself "the combination of molecules which form the cell." In the later which can also be designated as physiologic phenomena incorporates "the chemical changes, whether in the particles constituting the cell itself, or in the surrounding 'cytoblastema''. Thus Schwann more than 100 yrs. ago expressed our present point of view and because of this he is generally considered as "Father of modern cytology". The cell theory was quickly extended to unicellular organisms and it was recognized that protozoans are animals consisting a single cell. Then Haeckel divided the animal kingdom into two most important groups, *i. e.* Protozoa and Metazoa. In 1841, a famous Swiss anatomist, Albert Kolliker, applied the cell theory to embryology suggesting that sperms are histologic elements which originate in the organism and in 1844 he extended this concept to the ovum from which the organism develops by division of cells. In 1858, R. Virchow applied this theory to pathology and demonstrated that pathologic processes take place in the cells and tissues.

Protoplasm Theory—In the early years of nineteenth century, the attention of different scientists diverted to a "juice" or "*slime*" which had often been observed in the cells. This slime which we now know as the essential living substance, protoplasm was at that time thought to be only a byproduct of the cell. Even Schleiden, one of the founders of cell theory, spoke of the cell contents as a kind of "gum". Felix Dujardin (1835) was the first who recognized the importance of the cell contents. He studied the so called 'juice', tested its solubility and chemical properties and named it 'sarcode'. Sarcode, he defined as, "perfectly homogeneous, elastic, contractile, diaphanous gelatinous substance, insoluble in water and without traces of organization". Its full significance could not be made out

because he thought it to be present only in lower organisms. Robert Brown (1831) observed that each cell contains a small body which he called nucleus in the cells of orchid—a component found in all the cells. Hugo von Mohl (1846) found a similar content in plant tissue and named it protoplasm (Gr : *protos*=first+*plasma*=formation)—a term which was used in a very limited sense at an early time by Purkinje. It was von Mohl who proposed protoplasm to be common in all the living tissues.

Max Schultze (1861) established the similarity between sacrode and protoplasm of animal and plant cells, thus offering a theory which later was called by O. Hertwig (1892) the "Protoplasm Theory". This theory states that, "the cell is an accumulation of living substances or protoplasm, definitely limited in space and possessing a nucleus and a cell membrane".

Further the cells live in society, each to some degree depending upon other. To sum up, the activities of the organism are no more than the sum of the activities of its component cells. This theory, however, has come in much criticism because of the organisation theory which suggests that there are properties of the whole that are more than the sum of those of the parts. Prof. F. R. Lillie (1917) in a discussion of "Properties of the Whole' states, "if any radical conclusion from the immense amount of investigations of elementary phenomena of development be justified, this is : That the cells are subordinate to the organism, which produce them, and makes them large or small, of slow or rapid rate of division, causes them to divide, now in this direction, now in that, and in all respects so disposes them that the latent being comes to full expression ...... The organism is primary, not secondary; it is an individual, not by virtue of countless lesser individuals (the cells)."

Classical period of cytology—In the last quarter of the nineteenth century many fundamental discoveries were made. Due to this fact this period has been called as the "classic period" of cytology. In fact this was the period when much was contributed to the science of cell. The cell division attracted the attention of so many workers as a result the phenomena of amitosis or direct division (Remak, 1841) and mitosis or indirect division (Flemming) were reported. The latter is also called karyokinesis (Schleicher, 1879). Waldeyer (1890) proved that during mitosis, formation of nuclear filaments or chromosomes take place. Their equal division between the daughter nuclei or cells was suggested by Flemming, van Beneden,

Rabl. Hertwig (1875) reported that during fertilization, the fusion of the two pronuclei occur. Later on cell center (Boveri), the chondriome (Altmann, Benda) and the Golgi apparatus (Golgi) were discovered in the cytoplasm of the cell.

Not only that much was known about the cell but the technical advances for studying the cell were also made. The discovery of the better method of staining and fixing tissues made more easy to study the different activities of cell. Formerly only a few natural dyes (carmine and haematoxyline) were employed. Though these dyes are still extremely valuable, the coal-tar dyes were produced and used for the first time and this dye added greatly to the variety and effectiveness of staining procedures. Vastly improved section-cutting machines or microtomes were evolved. Compound microscopes were brought to a very high level of efficiency during this century.

During the closing years of the century several theories were formulated which gave directions to many of the investigations set to come. For example **Weismann** propounded theories of mechanism of individual development, heredity and evolution largely on the basis of what had recently been learnt about the behaviour of cells, nuclei and chromosomes throughout the life cycles of organisms.

Cytology in Twentieth century—In twentieth century, we have several new and extremely valuable technical and instrumental help for the detailed study of cytology. Methods for the successful cultivation of living animal and plant tissues under controlled conditions (tissue culture) have been devised. Direct study is also facilitated by the micro-manipulator, with which one can dissect or inject normal living cells. Another tool, for the cytological investigations, is the X-ray tube. Besides inducing alterations in chromosomes, X-ray have also revealed to ultramicroscopic structures of the plant cell. The chief advance in the microscopy is the invention of the electron microscope, which has a great resolving power, *i. e.* the ability to render fine details in an image. Autoradiography is another very important assistance to the cytologists in finding out biochemical pathways of different substances in the cells. Some of the staining and fixing techniques have been greatly altered and improved and some have been replaced by newer ones of greater value.

The most recent achievement in cell physiology and data on

current studies provide the means and ways to understand the different problems regarding the complex physiological activities going on inside the cell. Now the approaches are being made to cellular dynamics. It also lead to the more complex studies of respiration, irritability, growth and cell division. Biochemistry, also, has been applied to cell physiology. The Field of enzyme chemistry has had a spectacular development and the Nobel prize in 1955 went to Theorell for studies on enzymes of respiration in cell. Not only this but many enzymes and enzyme reactions in cellular activities have been studied by a large number of workers. The enzymes and co-enzymes concerned with these activities have been isolated and purified. There advances made in such a short time, present a prospect for the future which will be expected both interesting and exciting.

Today's accelerated pace of research, aided by new instruments, techniques, and point of views impart to biology a rapidly changing characters. All of us are quite aware, however, that each new and important discovery is not just a mere addition to our knowledge : it also throws our established beliefs into questions, and forces us constantly to reappraise and often to reshape the foundations upon which biology rests. For this purpose, the most significant and encouraging development in the twentieth century has been the alliance of cytology with the neighbouring fields of biology. As each field has extended its borders and have come into contact with those of other fields from which it acquires aid for its own further development. A brief account of such alliances between cytology and other fields of biology are discussed below.

(A) Cytology and Genetics—The most conspicuous and so far the most profitable of such alliances is between the cytology and the genetics. By the middle of the 19th century, Virchow proclaimed that the cell division is the central phenomenon in the reproduction of organisms. From this time onward actually starts the convergence between the study of cells and that of heredity and evolution. "Heredity appears as a consequence of the genetic continuity of the cells by division." The study of germ cells gave support to the "theory of continuity of the germ cells" proposed by Weismann (1833) to explain the transmission of heredity characters. According to this, heredity transmission is accomplished through the cells, found in the sex glands and not through the somatic cells. Further cell nucleus is the bearer of the physical basis of heredity as in it

the chromatin substance which constitute the chromosomes is contained. It is through these chromosomes the heredity transmission is made. In the beginning of 20th century the Mendel's laws of inheritance were rediscovered and shown to have a physical basis in the known behaviour of chromosomes through successive life cycles. Further McClung (1901-1902) suggested that sex determination was related to the chromosomes and in them too, the genes are the definite loci (Morgan) for the purpose. Thus we can speculate the close relationship between the two, *i. e.* cytology and genetics. From this convergence of the two **Cytogenetics** was born.

(B) **Cytology and Taxonomy**—It has been found that characters useful in classification can often be recognized in the number and form of the chromosomes. The chromosomal data not only aids in grouping of species or variety, but also furnishes strong suggestions as to the manner in which certain taxonomic units have arisen during the course of evolution.

(C) **Cytology and Pathology**—Since the diseases, particularly tissue diseases are primarily because of the abnormal activity in cells and tissues, the close relations between cytology on the one hand and pathology and medicines on the other hand should be expected. One needs only to mention the growth of cancer cells— a dreadful disease or the effects of viruses on cells structure and function to indicate the importance of co-operative studies on diseased tissues.

(D) **Cytology and Ecology**—It has been found that the study of chromosomes in related plants led us to speculate that the differences in chromosome number and form show significant correlation with differences in geographical range or ecological habitat.

(E) **Cytology and Evolution**—Since the chromosomes can be made to alter by treatment with some radiations (such as X-rays) and also some chemicals. These alterations manifest themselves in the morphology and physiology of the organisms ; it must indicate to some extent how the evolution has occurred.

What is Cytology—In the words of Sharp (1943) cytology can be defined as, "the branch of scientific biology that deals more or less directly with the structural and funtional organization of protoplasm, usually in single or closely associated protoplasts, and with the relation of this organisation to the phenomena of metabolism, growth, differentiation, heredity and evolution." He further says that "cytology is an integral part of biology and the future progress of science will depend very largely upon how well such integration is maintained."

2

THE CELL

The cell (protoplast of Sharp) is a structural and functional unit of the living matter. All the animals and plants are made up of a number of units, the cells which are intergrated for proper functioning. In unicellular animals (like Protozoa) and plants the cell unit is also the whole organism, but in multicellular animals and plants the cells are only minute parts of the whole organism, and organism as a whole dominates its parts. Whether a cell constitutes a whole organism or is merely one of the millions of minor units integrated to form a complex organism, it is a cell by definition.

The cell consists of a highly integrated and orgnised group of components performing specialized functions. The living cell is essentially a dynamic, self directed, and highly organized complex system of molecules and molecular aggregations which appropriates and utilize the energy of its sorrounding for the purposes of growth and reproduction.

The cell has been variously defined as, 'the unit of structure and function in animals and plants'; as 'the smallest living unit capable of independent existence'; as 'a small mass of living matter containing a nucleus or nuclear material.' The first definition fails because cells are not the only units of structure and function : the second was rejected as it does not take into consideration those filterable viruses seem to be living and are probably similar than true cells; and third does not serve the purpose as there are some plant cells, such as the blue green algae and bacteria, in which the protoplasm is not differentiated into nucleus and cytoplasm. The term cell is difficult to define as it represents an abstract generalization that attempts to cover too complex a field. It is possible, however, to give general description that will cover the majority of units of the cellular level of organisation and this has been done in this chapter.

The seemingly simple, but all important concept that the cell

is the unit of life is the culmination of centuries of study and researches by numerous investigators of different parts of the world. It is called the modern cell theory and explicitly states that all forms of life, plants, animals, and microbial are composed of cells (and their products) and arise only from pre-existing cells. The cell theory is a fundamental cornerstone of biology upon which all the biological sciences are based.

Historical Background—The observation on the cell or remains of cells magnified by the microscope were reported for the first time by the mid seventeenth century. Robert Hooke (1665), an English microscopist introduce the term cell in describing the honey comb-like structure of cork and other plant tissues. By the early nineteenth century a general pattern emerged, confirmed by such men as Lamarck (1809) in Germany and later Dutrochet (1824) in France and others, who independently stated that all plants and animals are composed of cells. This generalization was finally widely accepted and firmly established as the cell theory through the independently published works of German biologists, Schleiden and Schwann in 1838-1839. Mean while other important findings bearing on the nature of cell were being made. A few years earlier to the cell theory, Robert Brown (1831) reported the presence of a round distinct structure in cells, the nucleus. In 1839 Johannes Purkinje spoke of the living material of the cells as **protoplasm** which **Max Schultz** (*1861*) called the "physical basis of life". However, a major contribution to the cell theory was the principle advanced by **Rudolph Virchow** in 1858 that new cells arise by division from pre-existing cells. The modern cell theory recognizes the cells as the common structural units for all living things and which arise only from other living cells.

Further, the rapid development in the latter half of 19th century of preservation methods for cell structure provided a vast complicated details of cellular structure. The opening of the 20th century marked the beginning of the experimental approach and attempt to correlate cell structure with function. By the *1920S* the study of structure and function of cell components at the molecular level has been launched. The biochemical and biophysical approach together with the use of modern instrumentation, such as electron microscope and sophisticated chemical techniques, had led to a remarkable increase in our knowledge of the structure and function of the cells.

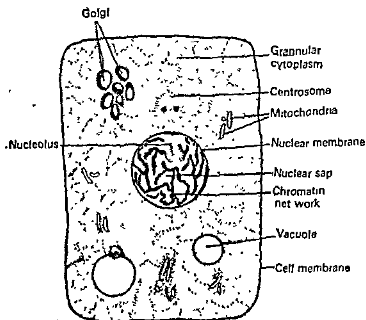


Fig. 1. Conventional structure of an animal cell.

Dimensions of cells—No one knows how small the smallest cell may be, for there are bacteria that are invisible under the highest power of microscope. The smallest cell, so far observed is the pleuropheumonia-like organisms such as *Mycoplasma gallisepticum* which measures 0·1 micron in diameter (1 micron=0·001 millimeter) and which produce respiratory disease in poultry. Inspite of this small size, it possesses all the necessary materials for conducting the life activities. There are, however, certain cells which are visible to the naked eye, indeed some are quite large. Thus a hen's egg is a signle cell, about an inch in diameter and that of an ostrich egg several times as large. Such cells, however, are large merely because they are gorged with yolk, an inert food substance upon which the embryo feeds until hatching. A single nerve cell, though of no great mass, may have an extraordinary length, in some cases being several feet long. Thus there exists variation in size of different kinds of cells, the cells of any given tissue of a particular organism are nearly uniform in size. The function of any particular kind of cell seems to have much to do with determining its size.

Shape—While the shapes of the cells are many and vary considerably, a typical cell shape, uninfluenced by inequalities of environment, pressure, specialized functions and relations is spherical.

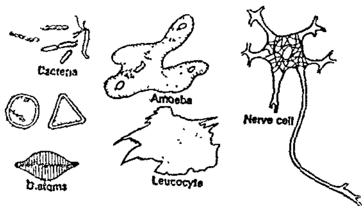


Fig. 2. Various types of cells.

However, it can be elliptical, spindle-shaped, cuboidal, polygonal, columnar, discoid, flat or plate-like, depending on the environmental and physiological conditions. Some of the cells have long branching fibres. Many cells have more or less stiff surface covering, the **pellicle** or **cortex**—that serves to hold the body in a permanent, though flexible shape. In spite of this, almost limitless variety of sizes and shapes are met with having an organisation dependent upon the possession of certain components necessary for the cell life.

STRUCTURE

The individual cells have their own architectural build up In very generalised cell, there is a limiting membrane in which the protoplasm is confined. This natural boundary is called the plasma membrane. Inside the membrane lies the protoplasm—the basis of life The **protoplasm** remains differentiated into an outer cytoplasm and the denser inner **nucleus** Thus the cytoplasm of the cell can be described as a portion of the protoplasm outside the nucleus and inside the plasma membrane This however forms the main bulk of the cell volume. If all the major particulates or organelles present in the cytoplasm are removed, then the left is known as **hyaloplasm.** The hyaloplasm is an aqueous substance in which many organic and inorganic particles are dissolved or suspended. The nucleus has its own natural membrane. It is supposed to control everyday function of the life.

Cell membrane—All cells, even those apparently naked, like *Amoeba* and leucocytes, have atleast a covering called the plasma membrane. It should not be confused with the cell wall which lies

outside the cytoplasm, sometimes separated from it by a considerable space. A cell wall is usually regarded as a protoplasmic product and is not believed to be truly living even though in some cases it s ems to be capable of growth.

The cell membrane is a double membrane of about 100A (A= Angstron unit) in thickness. It may however be connected with the nuclear membrane through the endoplasmic reticulum. Natural plant cell wall comprises three distinct layers, namely primary wall and the secondary wall, in between the two lies the third layer called lamella. The animal cell lacks the lamella, but instead an intracellular cement is secreted which like the lamella serves to bind the cells together. This cement however differs from the lamella both in origin and in its chemical composition.

It is no longer regarded as a limiting membrane for the cell and its different components but now it is believed to be a dynamic semipermeable barrier between the protoplasm inside and outside tissue fluid ; permitting the passage of solvents and some solutions while preventing the passage of colloidal material and of other substances in solution. It is also believed that there exists a 'carrier system' in association with the cell membrane which enables the osmotic "pump" to function. This carrier system is believed to be composed of enzymes secreted by the ribosomes.

(A) CYTOPLASM—The cytoplasm in the light microscope seems to be a hetrogeneous clear and viscid liquid. In most of the cells it remains differentiated into an outer ectoplasm and an inner endoplasm as in *Amoeba*. The ectoplasm is a clear gel with no granules while the endoplasm is granular and shows the movements. Other components or the organelles, found in the cytoplasm can be briefed as below. Their detailed descriptions are given separately.

1. Mitochondria—The mitochondria (singular=mitochondrion) [illegible] are among the largest [illegible] they appear as rod-like [illegible] in length and 5 micron in diameter. They however possess the changable shapes but can be recognised in the solution by their definite density. Their number vary considerably from 500 to 500,000 according to the species and the function type of cell. The recent cinomatographic studies on mitochondria [illegible] each having a [illegible] the cristae. It comprises an outer limiting membrane and an inner

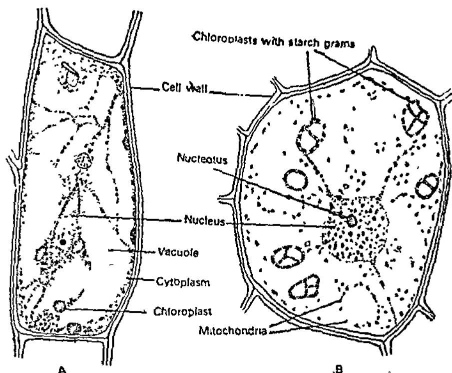


Fig. 3. Parenchymatous cells from the petiole of sugar-beat leaf (A) and from the stem of tobacco (B).

membrane. These membrances are very thin of about 60 to 70A ($A = 10^{-7}$). The space between the membranes is about 60A. These are similar to plasma membrane in chemical composition.

Chemically, the mitochondrion is made up of lipoproteins with protein 65% and the lipid about 35%. Sulphur also occur in SH-group. This SH-group may be present either in the protein or in the glutamine. Several vitamins have also been observed, the important ones are A, B_6, B_{12}, and C. With this ribofavine, folic acid and pantothenic acid with coenzyme A are also present in the mitochondria. The presence of all these substances indicate that [illegible]

[illegible] ber of unsolved problems regarding their morphology and physiology. Their presence can only be made out by the biochemical reactions.

Their average diameter varies from 0·4 to 0·8μ. They are usually spherical but may be irregular in shape, covered with a membrane of lipoprotein. Inside structure is always variable. In some of the cases they are found to be solid while in others they possess a dark outer zone and less dense core, and still in some others they possess cavities or vacuoles within the granular material.

Lysosomes produce hydrolysing enzymes, such as acid phosphatase, acid ribonuclease, acid deoxyribonuclease and cathepsins. But they do not have oxidative enzyme ; this property distinguishes them from the mitochondria.

3. Golgi bodies—Golgi bodies have been discovered in 1898 but controversies still exist as regards to their exact nature and function. Some authorities believe them as an artifect produced by osmophilic and orgentophilic substances, while others believe them an important organelle, taking part in the metabolism of cell. In fully functional cell, the Golgi bodies are well developed. They are composed of three main components, *i. e.* flattened sacs, large vacuoles, and clustures of small vesicles. In cells which show higher secretory activity, the Golgi complex is very much developed. The main function attributed to the complex has been historically related to the process of secretion.

4. Plastids—They are far more common in plant cells but are also found in a few types of animal cells, notably in some flagellate protozoons. Among plants, the most important type of plastid is the chloroplast, the green body that impart green colour to leaves
and other par [illegible] h
out of the ra [illegible] n
shape and siz [illegible] μ
in thickness. They may be spherical, ovoid depending upon the type of cell under study. Most of the available evidences indicate that they arise from the pre-existing plastids. They are transmitted to the daughter cells during division.

5. **Endoplasmic reticulum**—Embedded in the matrix of cytoplasm, are the membrane units called collectively, the endoplasmic reticulum (E. R.). It is found in all kinds of cells except the mature mammalian erythrocytes. The endoplasmic reticulum always exists in three forms, *i. e.* cisternae (lamellae), vesicles, and tubules. The cisternae are identified as rather long and flattened units, which are often arranged in parallel stocks. The vesicles are some what rounded in shape. The tubules are more diverse in shape than the

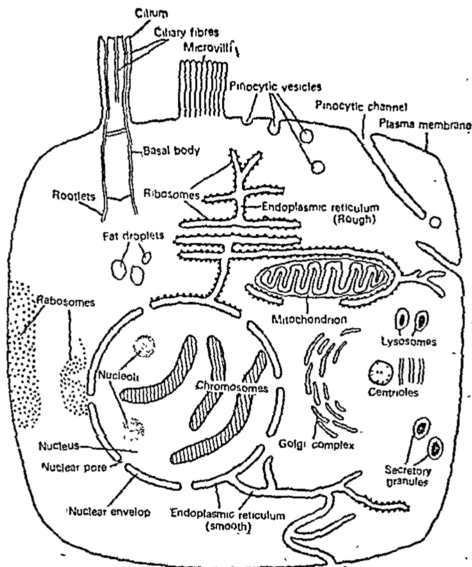


Fig 4. The structure of typical animal cell (Diagrammatic).

cisternae and vesicles. Endoplasmic reticulum performs many functions; the important ones are listed below :

1. The membrane provides an increased surface area in the cytoplasm for the metabolic activities.

2. The vaculoes bounded by the membrane permit the collection of products of synthetic activities of the cell.

3. There is a conservation of metabolic products due to the presence of vacuoles.

4. Its specialised arrangements such as sarcoplasmic reticulum may allow for the transmission of impulses or excitation intracellularly.

6. Vacuoles—There are spherical cavities filled with fluid substances of various kinds, each bounded by a delicate film. In some cells, these vacuoles are so conspicuous and large in number that the whole cytoplasm has a foamy or alveolar appearance. Vacuoles are particularly prominent in plant cells, and in protozoans among animals. In the cells of higher animals, they are not usually well developed.

7. Centrosphere—The term centrosphere is applied to that structure which lies at the focus of the astral rays during mitotic cell division. It is usually small and inactive during the so-called resting phase of the cell life but becomes active during the cell division. In resting stage, it comprises a sphere of more or less hyaline protoplasm in the centre of which lies one or two centrioles. Centrosphere lies close to nucleus usually, however it has also been observed considerably removed from nucleus and even within the nucleus.

(B) NUCLEUS—It is the most vital part of a cell which is invariably present in all the cells of higher animals and plants. However in lower organisms, distinct nucleus may be absent (such as bacteria, and certain flagellates). Normally one nucleus is present in a cell but more than one may also be present in certain cells. Such cells in plants are called the coenocytes and in animals the syncytia. Its position, is characteristic for each cell type and may the forced to assume peripheral position, as found in eggs having heavy yolk. Its shape and size are variable. It can be spherical, cylindrical, flattened depending on the type of cell.

[illegible]

of nucleus is filled with nuclear sap or karyolymph. In nuclear sap remain embedded other nuclear constituents. The sap is somewhat granular and homogeneous fluid, formed mainly of protein material. The notable other nuclear components are the chromatin, sex chromatin and the nucleolus.

1. **Chromatin**—The **chromatin** are the Feulgen positive mat rials in the interphase nucleus which during division becomes tl definite chromosomes. In short it represents the chromoson substance. Into this substance darker stained areas are observab which are called the **heterochromatin** ; in this beaded **chromomer** can be identified.

2. **Sex chromatin**—These are the specific heterochrometi bodies, found generally near the periphery of the nucleus. In nucleu usually one sex chromatin body is found but more than one can als be observed.

3. **Nucleolus**—**Nucleolus** *is relatively large, generally spherica* ball-like body. Its number in a nucleus vary in different cells an depend on either the species or the sets of chromosomes. It i supposed to be homogeneous structure but in reality it is formed o an amorphous part and a filamentous part. The former disappear and reappears during and after the cell division.

The chemical analysis of the nucleus suggest that it contains mainly the basic proteins, *i. e.* [illegible] proteins, *i. e.* tryptophan and [illegible] DNA and RNA ; lipids, mine[illegible] enzymes. No essential respiratory enzymes are found in nucleus. Nucleoside phosphorylase enzyme is worth mentioning as it is found only in nucleus and is involved in the synthesis of coenzyme DPN.-

A number of cell components or organelles have been discussed in brief, but still a number of problems remain unsolved regarding the morphology and biochemical nature. This provide an open challenge to the researchers. It is somewhat mysterious matter to the scientists ; inspite of their regular efforts, many facts are still unknown.

CHARACTERISTIC OF ANIMAL CELLS, PLANT CELLS, AND PROTISTS

By virture of their obvious differences in gross organisation and function, we can easily differenciate most higher animals from plants due to characteristically well organized systems in them like digestive, circulatory, nervous, and other systems which are usually capable of movement under their own power, and are completely dependent on performed organic substances for their energy and carbon supply. The higher plants contain the green pigment chlorophyll, which uniquely permits them to carry on photosynthesis, are usually immobile, and consist of their own characteristically orga-

However, an appreciable number of unicellular organisms exhibit characteristic of both plant and animals cells. As a consequence, some biologists classify them as plants and others place the very same organisms in the animal kingdom. While there are certain which can neither be called plants nor animals.They are known as protists. The evidence-suggests that at the lower level of the organization of living things, a sharp differenciation between plants and animals has not yet been occured. However, two separate lines of organisms that we cell plants and animals may well have arisen in the course of evolution from a now extinct ancient protist bearing a resemblance to certain present day species. Although all cells have a great many features in common, but there are certain cellular features that serve as the basis for classifying organisms in either plant or animal kingdom. The particular characteristics are reflected ultimately in the gross differences exhibited by two groups at the level of the cell or whole of the organism.

Unique Features of the Animal cell :

The centrosome, cilia and flagella—Virtually all animal cells and a number of lower and primitive plant cells contain a unique structure in the cytoplasm called the centrosome. It is usually present near the nucleus as a small clear region with radiating aster-like fibers and one or two deeply staining granules at its center called the **centrioles.** The cells of higher plants have no centrosome, but instead display two small clear areas during cell division called the **polar caps**, which apparently carry on the same function as the centrosome during cell division. The centrosome in addition to its primary role in cell division as described later in the chapter of cell division, somehow, controls the formation and activity of cilia and flagella, which are slender, filamentous, cytoplasmic structures projecting from the external surface of the cell membrane of certain cells.

Unique Feature of the Plant cell :

1. **The cell wall**—The cell wall is the most distinguishing characteristic of the plant cell. It is a moderately rigid envelop of inanimate material (external to the cell membrane) which entirely sorrounds each of the protoplasts. It [illegible] [illegible] ...al deposition.

2. [illegible] descrete cytoplasmic structures found in the cells of higher plant and certain unicellular

organisms but not in the cell of higher animals. They most often occur in spherical or disc-like bodies lying free in the cytoplasm.

3. Large-vacuoles —Large vacoles are characteristically found in the cytoplasm of mature plant cells and to a lesser extent in certain single-celled animals. Many kinds of animal cells, especially in fixed sections, display small vacuole, but these, however, occupy only a small volume of the cell and have a fleeting existance, appearing and disappearing at various time.

Regulative power of cells —The cells have the power of maintaining an integral consistency against the fluctuating conditions of out side. They possess the capacity to change them in response to the different external forces. The internal constancy is, however, controlled by the constant maintenance of the sodium and potassium *concentration independent to the outside variation.*

The Physiology of Cells

It is a common observation that a single fertilized cell grows to become adult animal in due course of time, e. g. man starts growing

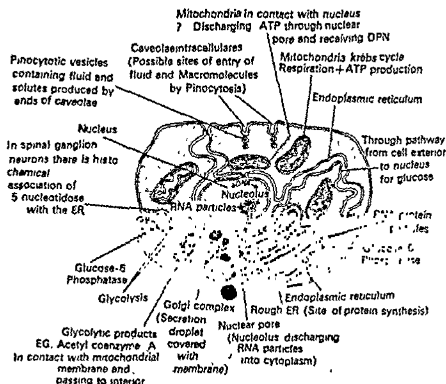


Fig. 5. Diagram depicting the division of labour in cell (Diagrammatic).

from a single cell, only 0·1 mm in diameter, but it increases in volume many thousand times, till the adulthood is attained. It is also known, that various activities are going on in the organism with the result it grows, reproduces, shows irritability and so on. All these activities originate from the basic unit structure of the body—the cell. All these functions or whatever the cell does is referred as physiology of cell.

The cell is not able to create matter, so the question arises, how does growth lake place ? From where does the material comes ? The organism take food, which is digested and absorbed, meaning thereby the matter comes from the food taken. The cell grows in size and divides and this phenomenon goes on indefinitely till the animal stops growing and dies. In addition to the utilization of food for growth, it is also used to liberate energy for performing different kinds of activities. The use of food as material for addition to living matter and for the liberation of energy to maintain the living state, is done apparently by some processes, infact innumerable chemical reactions, catalysed by the various enzymes. In fact, the living cells are the superfined laboratories in which even such reactions are going on which can not be successfully attempted by the chemists. For instance the chlorophyll containing cells of the plants are able to manufacture the organic foods in the presence of sun light by combining carbon-di-oxide and water but scientists have failed to probe successfully into the mechanism of this mysterious phenomena. The living matter represents a high degree of organisation,kept in a labile, steady-state condition by a continuous supply of free energy. At the same time a complex organisation is needed to funnel energy into channels useful for maintenance of organisation. Thus in conclusion it is most proper to say that energy is needed for the maintenance of organization which is needed for the proper utilization of energy.

The plant and animal cells resemble each other in all the essen tial make up except with respect to the ultimate source of energy upon which they rely. The animal ingests the food substances while plants use the light energy to manufacture carbohydrate, fats and proteins. Although carbohydrates, fats and proteins are not the immediate fuels for cell's machine ; instead, ATP (adenosine triphosphate) performs this function. The energy which is liberated by the oxidation or respiratory metabolism of carbohydrates, fats and proteins is utilized in a number of step to convert ADP (adenosine

diphosphate) to ATP. Thus it is easy to say that carbohydrates, fats and proteins are the 'crude fuels' which are used to generate the 'high grade' fuel, *i. e.* ATP, which in turn runs all the machines of the cells. Now we have to discuss what are these machines of the cell and what functions they perform.

1. Osmotic Work—It can be said safely that the living material differs greatly from its environment in the relative abundance and also in the absolute concentration of its component parts. The cells have to perform osmotic work to maintain this situation. For this, the cell is to perform both the functions—to accumulate materials found in the low concentration in its environment as well as to remove from within the materials found in high concentration in its surrounding medium. It is not enough, for the cell to accumulate or eliminate but it has to maintain the thermodynamically improbable situation also.

2. Electrical Work—The cell also performs electrical work. The plasma membrane of the cell is never equally permeable to specific anions and cations, nor they are ever accumulated to the same extent. By this reason all cells will exhibit a slight separation of change across their membrane, leaving the outside positive with respect to the inside or vice versa. This electrical difference is utilized in nerve cells for communication, by the transmission of an electric impulse in the form of a breakdown in charge separation.

3. Mechanical Work—It is very easy to understand that all the life is connected with motion of some sort whether it is the contraction of muscle cells, beat of cilia, the flow of the amoeba, the cyclosis of the protoplasm in a plant cell, or the movement of the chromosomes. *Motion requires energy funnelled into a machine that can convert chemical energy into work.*

4. Chemical Work—The cell has to carry out so many chemical functions in so many ways. The cell has to carry out the combustion and hydrolyse the carbohydrates, fats and proteins. Thus the cell must constantly replace components that have become altered during their functions. Furthermore, one of the major facts of life is that living matter always extends itself. The cell does not only repair itself, but is also duplicates. We do not have good understanding about this matter; either this is a physiological necessity or an evolutionary consequence of natural selection, but whatever the origin, the fact remains that a great deal of energy funnelled in to a cell is

utilized for growth and cell duplication. The concept of the hereditary material is necessary to the understanding of how the cell performs these vital functions.

It is a well admitted fact that the cell itself is not able to build all of its components from "scratch". A certain portion of the cell structure is retained as such from generation to generation. This portion of the material is called the hereditary material. The hereditary material plays the role in self duplication and also initiates the physiological functions that the cell performs. A great deal of negative organisation of the cell is located in the hereditary material, and the cell has to extend energy both in duplicating it is as well as in carrying out its directions regarding physiological function and growth.

The Transfer of Energy in the cell —The large number of activities of the cells are dependent on the transfer of energy. The transfer of energy leads to chemical reactions and physical changes. The chemical reactions may be endothermic or may be exothermic. An endothermic reaction always proceed only in the presence of energy. The exothermic reaction—is one that yields energy. Most of the oxidation that occur in the cell are exothermic. If the energy from exothermic reaction were simply liberated into the protoplasm, it would contribute little to the work of the cell. In other cases the energy must be transported; the energy is frequently carried in high energy phosphate bond, which when broken, liberate large amount of free energy. Phosphoric acid (H_3PO_4) can combine with many organic substances. In some cases the union gives rise to high energy bonds. In most cases the transportation and transfer of energy in the cell is done by ATP and ADP both of which are phosphate of adenosine, a nucleotide.

Besides these chemical functions the cell also produces enzymes for different activities which regulate the rates of the reactions in the living cell. The works of an enzyme can be understood by taking a simple example of the breakdown of maltose sugar in the digestive tract at the time of digestion.

In addition to these general catagories of energy-requiring activities, cells may engage in a number of other functions that are not quite so common in *all the cells*. *Cells interact and influence* with each other in the growth and development of an organism as well as in the physiological functioning of multicellular system. Furthermore from an evolutionary point of view most

significant recent development in the thermodynamics of life is that some of the organisation of the cell is tranrferred outside its cellular limits, that is, living system can utilize energy to organize their surroundings. This social phase of biological evolution occur most extensively at the organic and population level, and is most evident in human activities.

Thus a living system is a locus of behaviour and this behaviour has a structural basis. But ultimately it is the behaviour of the unit of life that comes closest to characterizing their functions.

SUMMARY

With rare exceptions all living forms are essentially made up of one or more basic units of structural compartments called the cells. The cell consists of a highly integrated and organized group of components performing specialized functions. As a unit of life cell can be described, as essentially a dynamic, self directed and highly organized complex system of molecules and molecular aggregations. The cell is delimited by the semi-permeable membrane. It contains a very prominent spherical structure termed the **nucleus**. The cell also contain **organelles** (the structures of definite functions)and **inclusions** (the structures which are thought not to have specific functions.) The cells vary in shapes and sizes.

The cell contains **protoplasm**. The protoplasm of the nucleus is termed as **nucleoplasm** and that in the remainder of the cell as **cytoplasm**. The nucleus has its own membrane which delimits it from the rest of the cell. **Chromatin** within the nucleus, become differenciated into discrete **chromosomes** at the time of cell division. Important structures characteristic of many cells include, [illegible] structure which [illegible] the power plant [illegible] a intracellular pump ; the centrosome with its centriole which take part in cell division ; and the lysosomes, rich in hydrolytic enzymes. The cilia and flagella of the cell serve to propel the cell.

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energy. This however, occurs in smoth muscle cells which produces the movement to the intestine and other parts of the body. But in the life of the cell there are many other processes besides the more building up and making down the substances. Many substances, specially the aminoacids, tend to be a constant state of flux, changing from one carbon frame work to another by complicated intermedidate reactions.

Many of the reactions that take place in cell metabolism occur in cycles. This means that there occurs a series of reactions in which a substance may be transformed and returned to its original state. Such cycles, when they occur in the cell, usually involves the transfer of energy. Such cycles include the chlorphyll cycle in the photo. synthesis and the citric acid cycle in cell respiration—

ANABOLIC PROCESSES

A cell, consist of chemical substances that are for the most part continually changing and are united and organised by chemical processes. Chemical reactions in which simpler substances are combined to form more complex substances are termed synthetic processes.

PHOTOSYNTHESIS

In all the process in nature, photosynthesis is perhaps the most fundamental. It is almost universal method by which energy is obtained from the inorganic world, *i. e.* the absorption of sunlight and the conversion of this to chemical energy in the form of carbohydrates. This process only occurs in the cells that contain chlorophyll. The carbohydrates so build, directly or indirectly serve as the source of free energy of all living things. The carbohydrates manufactured in photosynthesis are used for the synthesis of all other organic compounds found in the cell, In this process, a portion of the carbohydrate is oxidized to provide the energy for the sythetic reactions which also use the carbohydrates as raw materials.

The process of photosynthesis is esentially the same in all green plants and also in all green cells. In most higher plants the leaves are the chief organs where photosynthesis occur but in some cases the stem may store this function in many herbs or take it over entirely as in the cactus family. In most plants, the leaves arrange themselves in such a fashion so that they can get as much light as they can. Ordinary white light, such as that which comes from

the sun, is the mixture of light of various colours. Leaves are green, because they reflect green light. They also contain certain amount of yellow and blue light, which usually when mixed, give green impression. The red, orange, some of the yellow and most of the blue go into the leaf and are absorbed by it, Maximum photosythesis occurs in the wave lengths of the blue and red portions of the spectrum.

In most of the cases, there are only two important ways by which the energy become available to the cell: radiant and chemical. Radiant energy in the form of light, *i. e.* traceable to the sun. The radiant energy is in the form of chemical compound that can yield energy. The chemical energy available to the cell from the inorganic world is extremely limited both in kind and in quantity. In certain bacteria, the energy is obtained in this way.

In this way, practically all the cells, including the nonchlorophyll-containing cells of plants and bacteria, and all animal cells are dependent for the energy for their vital activities upon organic substance manufactured by the green cell or chlorophyll containing cells. The carbohydrates manufactured in this process are used for the synthesis of all other organic compounds found in the cell.

In this way, once the light energy has been absorbed and used to synthesize carbohydrate, any further energy that becomes available in the cell comes form the breakdown of the carbohydrates or other organic compounds formed from them. Further, since photosynthesis is practically the only way through which the element carbon passes from the inorganic to the organic world,therefore all cells are directly or indirectly dependent on photosynthesis for this element.

The function of pigments in photosynthesis—Chlorophyll is present in all photosynthetic cells. Several varieties of chlorophyll have been identified, all have the same essential porphyrin structure, or tetrapyrrole nucleus. Magnesium, in nonionic form, is present in the nucleus and is attached to the end of the pyrroles. In chemical terms, chlorophyll is methyl phytol ester of the parent dicarboxylic acid, the chlorophyllin. Phytol is a long chain alcohol, containing one double bond. It is a derivative from vitamin A by dehydrogenation. The various chlorophylls differ from one another only in the side chains attached to the other ends of the tetrapyrrole nucleus.

Four different kinds of chlorophyll are now known, and each

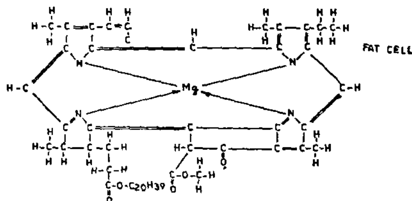


Fig. 6. The structural formula of chlorophyll.

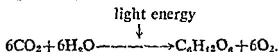
is identified by a letter of the alphabet *a*, *b*, *c* and *d*. The chlorophyll *a* is believed to be present in all photosynthetic plants. In green algae, bryophytes, and tracheophytes, there is also some chlorophyll *b*. In the diatoms and brown algae, chlorophyll *c* occurs instead of *b*. In the red algae, chlorophyll *d* is present. Chlorophyll molecules are quite complex ; the formula for chlorophyll *a* stand $C_{55}H_{72}O_5N_4Mg$. The large number of atoms is arranged in the form of a complicated ring, with a single Mg atom in the center and a long tail. Very little is however known about how an organism forms these complex chlorophyll molecules. It has been observed by some plant physiologists that they are not produced in large quantity unless the plant is exposed to light. The manufacture of chlorophyll always occur inside the chloroplast, except blue green algae, which possess no chloroplasts.

In all plant cells and in the purple sulphur bacteria (which are capable of photosynthesis), carotenoids (yellowish pigments) are present. There is some question, however, that carotenoids are present in the green sulphur bacteria (also capable of photosynthesis). The carotenoids are essentially hydrocarbons and are usually of two types, *i. e.* carotenes and xanthophylls.

The chlorophyll is organised in submicroscopic disc-shaped bodies called grana with the chloroplast. Chlorophyll, nucleoprotein, and enzymes seems to be structually integrated in the grana. With some exceptions, chlorophyll is manufactured by the plant from another substance, protochlorophyll. This occurs only in the presence of light. Several experiments using short exposures to light followed by an analysis of the results have led us to have clear understanding

of the role of chlorophyll. Two important different and distinct types of reactions occur. One of these is dependent on light and is called the photochemical or light reaction. The other reactions are independent to light as such they can also occur in the dark ; though they do not require darkness they have been called the dark reactions.

The Biochemistry of Photosynthesis—The over-all reaction which take place in photosynthesis might be writen as :



$$6CO_2+6H_2O \xrightarrow{\text{light energy}} C_6H_{12}O_6+6O_2.$$

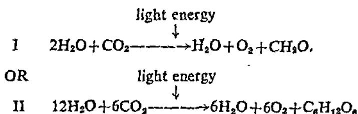
Here $C_6H_{12}O_6$ represents the carbohydrate. In some plant cells *such as diatoms, fats appear as a result of photosynthesis and some* investigators have suggested that perhaps fats might be the first products of photosynthesis. But, however, the photosynthetic quotient in diatoms has a value of one(that of fat synthesis is greater than 1), and the oil never accumulates except in the cells which have ceased growing. In most plant cell, the fat is a food reserve, as in animal cell.

Van Neil (1931) first of all suggested that water is a hydrogen donar in the oxidation-reduction process that occur during photosynthesis. He has noted this when he has observed the reaction which occur in photosynthetic sulphur bacteria.



$$2H_2S+CO_2 \rightarrow 2H_2O+S+CH_2O.$$

In this way in the green plant cell H_2O appears to be the hydrogen donor playing the role similar to be that of H_2S, and by analogy, the above equation may be written as follows :



$$\text{I} \quad 2H_2O+CO_2 \xrightarrow{\text{light energy}} H_2O+O_2+CH_2O.$$

OR

$$\text{II} \quad 12H_2O+6CO_2 \xrightarrow{\text{light energy}} 6H_2O+6O_2+C_6H_{12}O_6$$

Ruben et al provided the convincing proof of the validity of Van Neil's argument by using tracing isotope element. They used the heavy oxygen atom, O^{18}. This isotope, can be distinguished from the normal oxygen O^{16} with an instrument called the mass spectrometer. In 1941, Samuel Roben and Martin Kamen exposed the plant in carbondioxide having O^{18}. But all the oxygen given off

by the photosynthezing plant was O^{16} but the sugar that formed in the plant contained O^{18}. They further exposed another plants to ordinary CO_2 but supplied them with water having O^{18}. This time, the oxygen gives off, was identified as O^{18}. This is quite clear here that oxygen only come from water not from carbondioxide, or the hydrogen donor is the water and the equation has modified like No. II.

Certain bacteria capable of photosynthesis use variety of hydrogen donors including H_2S and also various organic compounds. But the organisms using organic compound as hydrogen donor are primitive ones.

The light reaction or Photochemical reaction in Photosynthesis— During the past twenty years much progress has been made in learning the biochemistry of photosynthesis. However, several lines of experiments suggest that thermochemical reactions follow the photochemical reaction.

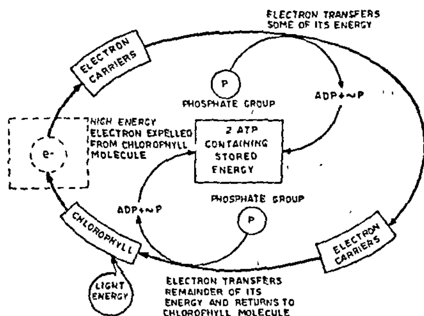
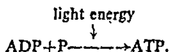


Fig. 7. The ~~cyclic part of the~~ light reaction in photosynthesis.

In the light reaction, the light energy is however, absorbed by cell pigments chlorophyll and is transformed into chemical energy and temporarily stored into compounds. The first compound is the adenosine triphosphate (ATP) and the second compound is *triphosphopyridine nucleotide* (TPN). ATP is formed by the addition of a phosphate group to adenosine diphosphate (ADP). To attach

this third phosphate group to ADP, energy must be supplied. In photosynthesis this energy comes from light.



$$ADP + P \xrightarrow{\text{light energy}} ATP.$$

Further, present observations on the problem indicate that ATP is formed in two different ways within the chloroplasts. In the first, a cyclic set of reaction, light is absorbed by a molecule of chlorophyll, causing an electron to be expelled from the molecule. This high-energy electron passes along a series of electron carrier substances that can take on and transfer electrons. As the electron

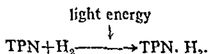
12 MOLECULES OF TPN-H2 CONTAINING STORED ENERGY
TPN
TWO OF THESE ELECTRONS
TWO OF THESE HYDROGEN IONS
H+ HYDROGEN ION
e- HIGH ENERGY ELECTRON EXPELLED FROM CHLOROPHYLL MOLECULE
WATER MOLECULE IONIZED
H2O
12 MOLECULES OF ATP CONTAINING STORED ENERGY
24 MOLECULES OF WATER ENTER REACTION
OH- HYDROXYL ION
CHLOROPHYLL
ADP+~P
PHOSPHATE GROUP
P
PIGMENT
24 OH RADICALS COMBINE TO RELEASE
ELECTRON CARRIERS
e-
LIGHT ENERGY
ELECTRON TRANSFERS ENERGY AND CONTINUES ON TO REPLACE ELECTRON ORIGINALLY EXPELLED FROM CHLOROPHYLL A MOLECULE BY LIGHT
HIGH ENERGY ELECTRON EXPELLED FROM PIGMENT
LIGHT ENERGY
12H2O 6O2

Fig. 8. The noncyclic part of the light reactions in photosynthesis.

moves along this series, its energy is transferred to enzyme system that catalyze the change of ADP to ATP. Before the electron returns to its chlorophyll molecule, two or more molecules of ATP have been formed.

However, in the second of the two ways, a noncyclic set of reactions take place in which both ATP and $TPN.H_2$ are formed. Water is however, involves in such reactions ; and it supplies the hydrogen that combines with TPN to form $TPN.H_2$. In this

reaction also the energy is derived from the light. The chemical reaction take place as follows.



$$TPN + H_2 \xrightarrow{\text{light energy}} TPN.H_2.$$

In this way the light absorbed by a certain pigment molecule causes a high energy electron to be expelled. The pigment has not yet been identified but it is supposed that it is either chlorophyll *b* or one of the yellow pigments present inside the chloroplast. Almost now all the plant physiologists believe that as the electron leaves the pigment molecule, it is replaced by an electron from a *hydroxyl* ion (OH^-) obtained by the ionization of water. The high energy electron from the pigment is picked up by electron carrier. Light is also absorbed at the same time by the chlorophyll *a* molecule, and thus a second high energy electron is expelled. The electron carriers then transfer the first high energy electron to this chlorophyll molecule. As soon as the electron however, moves to the chlorophyll molecule, its energy goes into the formation of an ATP from ADP.

It is however, noticeable from that this complete chain of reaction, light energy is absorbed at one point by the pigment molecule and at the second point by chlorophyll *a* molecule. Thus two high energy electrons expelled from chlorophyll molecules and two hydrogen ions (H^+) from ionised water join a molecule of TPN and thus formed $TPN.H_2$. The light energy which is absorbed at this time by the chlorophyll molecule has now been transferred to the $TPN.H_2$. For every twenty four molecules of water entering this noncyclic set of reactions, twelve molecules of ATP and 12 molecules of $TPN.H_2$ are formed. It should be kept in mind, however, when each of the OH^- ions derived from the water loses an electron, it becomes an OH radical. Twenty four of these radicals combine to form 12 molecules of water and 6 molecules of oxygen. This is that oxygen, *i. e.* given off during photosynthetic activity.

In this way the "light reaction" consist of the absorption of light energy by chloroplasts. It changes it into chemical energy. This energy holds by molecules of ATP and $TPN.H_2$. The simple reaction occurs as below.

LIGHT REACTION

(I) $2H_2O \nearrow 2(H^+)$; $\searrow 2(OH) \longrightarrow H_2O + O$ released off both.

(II) $TPN + 2(H^+) \longrightarrow TPN.(H^+)_2$

(III) $2ADP + 2$ phosphate $\longrightarrow 2ATP$.

The dark reaction or Thermochemical reaction in Photosynthesis—Several methods have been found largely responsible for the successful analysis of the thermochemical "dark" reactions in photosynthesis. The most basic result of the "dark reaction" is the formation of multicarbon molecules containing chemical energy which can be generally used by the cell at a later time. In other words, it can be said as the formation of foods. More than twenty different reactions are known to occur in the cyclic process. The carbon source

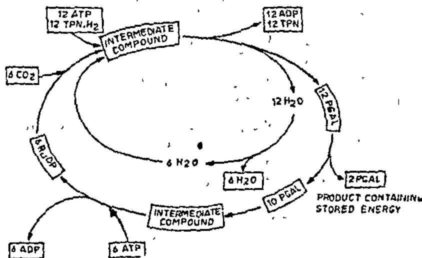


Fig. 9. The dark reaction in photosynthesis.

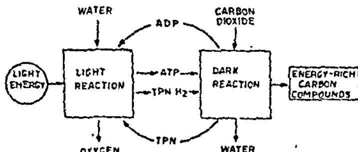


Fig. 10. Summary of photosynthesis.

is carbon-dioxide. In a series of steps, carbon-dioxide is combined with a complex molecule of ribulose diphosphate (RuDP) or (RDP).

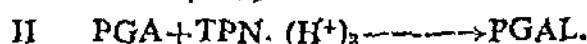
This however eventually results in a 3-carbon molecule phosphoglyceraldehyde (PGAL). For every six molecules CO_2 taken up by the cell, 12 molecules of PGAL are formed. The 10 molecules, out of the 12, are again cycled back into a series of reactions that produce RuDP and thus only two molecules of PGAL are left to form carbohydrates and all the other multicarbon compounds in the cells. This PGAL then undergoes a rearrangement of its elemental structure to form glucose.

For doing all this, there must be a supply of energy. This energy is of course available from the compound that formed during the "light reaction", *i. e.* the ATP and $TPN.H_2$. As the energy is released from these two compound for dark reaction, they again form ADP and TPN respectively. In this way the ATP and $TPN.H_2$ act as a carriers of energy. The over all process which take place during dark reaction is as follows.

DARK REACTION



I RuDP+CO_2———→PGA



II PGA+TPN. $(H^+)_2$———→PGAL.



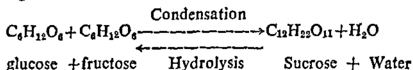
(III) PGAL———→Glucose.

Because of the simplicity, some worker considered the possibility that photosynthesis is a reversal of the kreb's cycle that the hydrogen made available from water during photosynthesis might be used to reverse each step in the dehydrogenation and deoxycarboxylation occuring in respiration. The reactions of the krebs cycle are known to be reversible in both plant and animal cells and the reverse of decarboxylation is the fixation of carbondioxide and reverse of dehydrogenation is reduction. However, experimental data are not in keeping with the explanation, but this reversal of the krebs cycle occur in the plant cells most conspicuously in the dark.

Carbohydrate synthesis—Carbohydrates contain only carbon, hydrogen and oxygen ; the hydrogen and oxygen are in the same ratio as they are in water. The formation of glucose or monosaccharides sugar have been shown in the process of photosynthesis.

The disaccharide molecule is formed by the combination of two monosaccharide molecules with the liberation of one molecule of water, the process being called condensation. The disaccharide molecule may also be split into the two component sugar molecules, by absorbing the water molecule. The process is called hydrolysis.

Thus the molecule of sucrose, $C_{12}H_{22}O_{11}$, is formed from one molecule of glucose and one molecule of fructose.



Maltose and lactose are two other disaccharides.

Maltose or malt sugar is found in germinating seeds and grains and also as a product of the degestion of starch. Hydrolysis of maltose shows that its molecule is formed from two glucose units. The lactose found in the milk of mammals including man. All of the above disaccharides have the same molecular formula, $C_{12}H_{22}O_{11}$, but of course with different graphic formulae.

The polysaccharide molecule is made up of many simple sugar units joined in long chains, with the general molecular formula $(C_6H_{10}O_5)n$ where *n* is the number of monosaccharide units. The most important are starch, glycogen and cellulose. Starch molecule is composed of hundreds of glucose units joined to form a long chain from which extend similar side chains. The chains are believed to be flexible and are able to fold upon themselves so as to form conpact molecules. The glycogen molecule is smaller than that of starch and also composed of small chains of glucose. This is generally formed in animal cells. Cellulose is the main constituent of the walls of plant cells. This is also formed by the combination of starch.

Formerly it was thought, that the larger carbohydrate molecules of polysaccharides were formed from monosaccharides by the condensation process. But the present knowledge on the subject pointed out that the synthesis is more complicated, that glucose phosphate is involved in the process as is clear by the process of glycogen formation (a characteristic polysaccharide sugars found stored in human liver) from the monosaccharide sugars, *i. e.* glucose, glactose and fructose.

Synthesis of Glycogen—Before glucose is converted into glycogen it first react with phosphoric acid to form glucose-6-phosphate. The phosphorylation of glucose is brought about by an enzyme known as hexakinase and a compound called adenosine triphosphate (ATP). ATP, however, furnishes the phosphoric acid for the phosphorylation and becomes adenosine diphosphate (ADP). After glucose-6-phosphate is formed, the enzyme phospho-glucomutase transfer the phosphate group from carbon 6 to carbon 1, producing

glucose-1 phosphate. In the last, phosphorylase however, convert many molecules of glucose-1-phosphate into glycogen and phosphoric acid. In the same way, the glycogen synthesis also take place

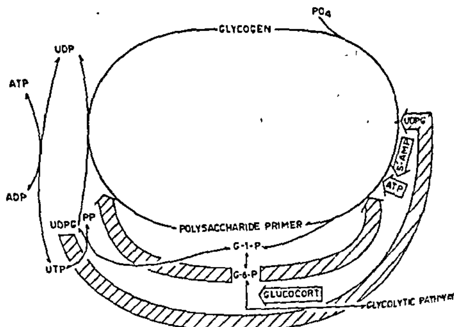


Fig 11. Pathways of regulatory factor in glycogen metabolism.

Fig. 12. The phosphorylation of different sugars.

from the glactose fructose and mannose sugars as is clear from the flowing chemical reactions.

The formation of glycogen from glucose-1-phosphate is a bit complex in chemical reaction and nature. Under the influence of a pyro-phosphorylase, glucose-1-phosphate reacts with uridine triphosphate to form uridinediphosphate glucose (UDPG), an intermediate product, which is also important in the pathways leading to glactose glucoronic acid, and mucopolysaccharides. UDPG—glycogen glucosyl—transferase (commonly known as "glycogen synthetase") transfer the glycose-moieties of UDPG to the free carbon 4 positions at the non-reducing terminal of pre-existing polysaccharidic chain. A "primer" of branched polysaccharide, the main linkage of which are α-1,4 is essential for the reaction. The equilibrium constant of this reaction favours the synthesis of glycogen. UDP, by inter action with ATP, is converted to UTP,so that the uridine nucleotide can take part in the pyrophosphorylase and transferase reactions in the cyclic manner. In the absence of other enzymes, linear, α-1, 4-linked polysaccharides are synthesized, similar to the amylose fraction of starch. More over the branching enzyme (amylo—(1,4—1,6)—transglucosidase) is required for the formation of the highly ramified glycogen molecule. The liver cells are modified to synthesize the glycogen (sometimes known as animal starch) from glucose, fructose, and glactose. Some, the little quantity of glycogen is also formed by the blood cells.

Not only this but certain cells of the body are modified to convert the body or food proteins and fats into the different carbohydrates. This type of the synthesis of carbohydrate will be dealt-in preceding account. There is very definite evidence that glucose may arise from protein and to some extent from fat.

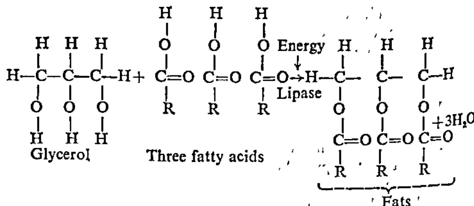
Fat or Lipid metabolism—Like carbohydrates, fats are composed of carbon, hydrogen, and oxygen atoms only, but in fats the ratio of hydrogen and oxygen atom is always greater than 2 to 1. The oils found in the living cell are chemically the same class of substance as fats.

Two classes of substances are involved in building a fat, *i. e.* glycerol and fatty acids.

Glycerol (glycerin) is perhaps best known as an ingredient of candies and cough medicines. Glycerol is a 3-carbon molecule which can be formed by glucose. The acetic acid is the simplest of the fatty acids. Acetic acid CH_3-COOH is a 2 carbon molecule

that is formed during the breakdown of glucose. Other fatty acids are built up from acetic acid, so they usually have an even number of carbon atoms. The ones, most commonly used in building fats have from 14 to 18 carbon atoms. The—COOH radical is characteristic of the molecular structure of the organic acids.

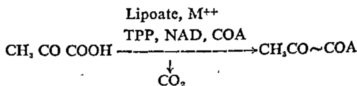
The—COOH radical of a fatty acid can react with the—OH radicals of glycerol as follows.



As in the synthesis of carbohydrates, water molecules are split off in the process; it is a dehydration synthesis and both energy and enzyme are required. The enzyme is required to catalize the reaction.

A number of classes of substances are chemically related to fats. All are formed from fatty acids, but in combination with substances other than glycerol.

Chemically, the carbohydrate is the major raw material for the synthesis of fatty acids. Pyruvate, by means of oxidative decarboxylation, forms "active acetate" acetyl-COA :



Metabolic pathways are also available for the synthesis of fatty acids from amin oacids. The glucogenic aminoacids are converta bleto pyruvate, the ketogenic aminoacids from acetate or acetoacetate, both of which are lipogenic. The acetyl—COA is the immediate starting material for the formation of fatty acids. The anabolic pathways of fatty acid synthesis are found both within or

without mitochondria. The extra mitochondrial pathway is as follows.

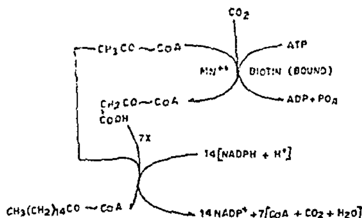


Fig. 13. Extra mitochondrial pathway for fatty acid synthesis.

It is clear from the figure that in the first step, an example of CO_2 assimilation, acetyl-CoA is converted to malonyl-CoA,ATP providing the energy for formation of the carbon-carbon linkage. Biotin is the cofactor for this reaction, functioning as a carrier for the CO_2. In the over all conversion, a molecule of acetyl-CoA condensed with seven molecule of malonyl-CoA in a series of reactions comprising decarboxylation, dehydration. The equipment of double reduction, forms palmityl CoA. This pathway is favoured by the high NADPH/NADP ratio obtaining in the cytoplasm outside the mitochondria, particularly in tissue such as liver and adipose tissue, in which the pentose shunt is present, an important source of NADPH.

Further, malonyl-CoA undergoes a transesterification from coenzyme A to an enzyme bound thiol group. Then acetyl-CoA, which is destined to provide the two methyl terminal carbon atoms of fatty acid, condenses with the malonyl-enzyme, CO_2 is lost, and acetoacetyl enzyme is formed.

Synthesis of Protein—The basic building units of protein molecules are aminoacids. They contain carbon, hydrogen, oxygen and nitrogen. The simplest aminoacid, glycine, can be obtained from the simplest fatty acid, acetic acid. This is done by substituting an amino radical,—NH_2 for a hydrogen atom.

Approximately twenty different aminoacids occur in the

proteins. Apparently most green plants can synthesize all of these from simple materials. Animal on the other hand must obtain aminoacids ready-made in their food, though many animals can change one kind of acid to another within the cell. Our own cells can form about ten aminoacids in this manner. Once the necessary aminoacids are made or obtained, the synthesis of protein in a matter of linking the aminoacids together. The whole process of protein synthesis include the following steps.

1. Mechanism of formation of the peptide linkage.
2. Specification of the primary level of organisation (amino-acids sequence.
3. Specification of the three-dimentional conformation of the protein secondary, tartiary and quaternary levels.
4. Mechanism of attachment of prosthetic group.
5. Mechanism of regulation.

The over all reaction which take place in the protein synthesis is as follows.

$$R_1-\overset{\overset{NH_2}{|}}{C}-COOH+R_2-\overset{\overset{NH_2}{|}}{CH}-COOH\xrightarrow{enzyme}\underbrace{R_1-\overset{\overset{NH_2}{|}}{CH}-\overset{\overset{O}{\|}}{C}-NH-\overset{\overset{R_2}{|}}{CH}-COOH}_{dipeptide}+H_2O$$

In the same way tripeptides may be formed as follows.

$$\begin{matrix}H\\ \diagdown\\ \diagup\\ H\end{matrix}N-\overset{\overset{H}{|}}{C}-\overset{\overset{O}{\|}}{C}-\boxed{OH\ \overset{H}{+}\ \underset{H}{}}N-\overset{\overset{H}{|}}{C}-\overset{\overset{O}{\|}}{C}-\boxed{OH+\ \overset{H}{}\ \underset{H}{}}N-\overset{\overset{H}{|}}{C}-\overset{\overset{O}{\|}}{C}-OH\xrightarrow{enzyme}$$

$$\underbrace{\begin{matrix}H\\ \diagdown\\ \diagup\\ H\end{matrix}N-\underset{\underset{R}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-\underset{\underset{R}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}-N-C-C-OH+2H_2O}_{Tripeptide}$$

The template theory seems more probable to indicate the complete process. The general treatment to this synthesis will be given here, and for more details student are advised to consult the chapter

on protein synthesis. According to this theory the template determines the sequence of aminoacids in which they are to be arranged in protein molecule. Spaced along the template is a series of loci. At each locus a specific aminoacid becomes temporarily attached to the template. The aminoacids thus attached on the template become united by peptide bonds, thus giving rise the protein molecule. The protein is than detected from the template which is then available for the formation of another protein molecule of the same kind. The loci of the template to be activated by combining with phosphates obtained from ATP. These phosphate group is then replaced by aminoacids which become loosely attached to the template by their carboxyl groups. Thus the energy for protein synthesis would be supplied by ATP. The different kinds of RNA take part in the synthesis of protein. Deoxyribonucleic acid (DNA), which is found in the chromosomes believe to prepare the template or act as template, and this provide a reason why the protein constitution of organism is inherited.

Nucleic acid Synthesis—In (1920) it becomes clear that much of the dark-staining material in the nucleus of the cell is composed of a class of material called nucleic acid. The chemical units in nucleic acids are rather more complicated than in the simple sugars, fatty acids, glycerol and aminoacids. The chemical mechanism by which nucleic acids are formed, however, is the same—dehydration synthesis.

There are two different series of nucleic acids **ribose nucleic acids** (RNA) and **deoxyribose nucleic acids** (DNA). The RNA series is built from units which contain ribose, a 5-carbon sugar ; the DNA series is built from units containing deoxyribose, another 5-carbon sugar that differs from ribose in having one less atom of oxygen. In the units from which nucleic acids are synthesized, ribose or deoxyribose is attached, on the one hand, to the phosphate group ($^-PO_4$) and, on the other, to one of the five different kinds of carbon nitrogen structures called bases.

Further, the ribose appears to arise from glucose partly via anaerobic pathway involving, essentially oxidative decarboxylation of the phosphorylated hexonic acid.

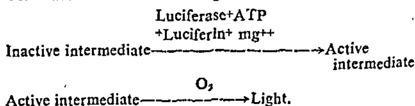
Such a molecule which is formed by the pentose sugar, base, and phosphate is called a nucleotide. However, the nucleotides that unite to form nucleic acids have only one phosphate group instead of the three present in ATP. The detailed synthesis and

process of synthesis of nucleic acids have been discuss in the chapter of nucleic acids synthesis.

BIOLUMINESCENCE.

Plants are capable of utilizing radient energy for biosynthesis from the sun or by some other source. But in many cells, a reverse phenomena however occurs and they usually emit-light by the chemical reaction. This emission of the light by a living organism is termed as **bioluminescence.** Luminescence cells are found in animals and plants both, but in the case of latter they are rare and generally very weak. In the animal cells they are far more common and are more spectacular.

Mechanism of luminescence—Luminescence is thought to occur due to the oxidation of a substance known as **luciferin.** Luciferin name was given to this substance by **Emil Dubois** (1885). This reaction is however catalyzed by the enzyme known as luciferase. Luciferin is apparently stored as granules which must first be broken down before the luminescent reaction can proceed. But the mechanism by which this occurs is not known. It is however interesting that the mixture of luciferin from one organism with laciferase from another is without effect, *i. e.* no luminescence results, unless the two organisms are closely related. It has been noted that the luminescence in the firefly is however, controlled by regulating the admission of oxygen to the reactive substances. Thus it is believed that the following reactions occur.



When oxygen is admitted, there is a flash of light. But whether luminescence is controlled by regulating oxygen admission or by some other mechanism is not certain. It is thought possible that the nervous control over luminescence is similar to its control of muscle contraction. If it is so, the liberation of ATP is the key mechanisms to do so.

The nature of bioluminescence—The absolute candle power of luminous organisms is low, ranging from 1/50 to 1/400 candle. The colour of the light varies with most luminescence occuring in the orange-yellow-green-blue ranges.

Function—Luminescence is thought to serve three function (1) Sexual attraction, (2) protection by blinding, and (3 attraction of prey. Finally in certain cases, luminescence serves n purpose, but merely a by product of metabolic processes.

CATABOLIC PROCESSES

Most of the catabolic processes that occur, in the cells in volve the oxidation of different organic compounds such as car bohydrate, proteins and fats. The oxidations are promoted b enzymes and yield energy which is utilized in the cell for all activitie The term respiration is frequently used to cover the entire series c reactions involved in the degradation of carbohydrates, fats, an proteins by a series of oxidations. The energy which is supplie during the degradation is not only enough for the reactions to procee but also for the synthesis of compounds with high energy phosphat bonds. The compounds represent powerhouse from which the energ is used for various cellular activities including : (1) metabolic pro cesses, (2) contraction, (3) active transport of substances into an out of the cells, and (4) impulse propagation.

In the multicellular animals there are generally three phase involved in a series of reactions by which complex food-stuffs ar utilized by an organism. In the phase first the complex molecule of carbohydrate, fat, and protein are split. The basic reactio involved the addition of water and thus called **hydrolysis**. Enzyme also participate in these reactions. Those enzymes which howeve bring about the hydrolysis are collectively called as **hydrolases**.

Hydrolysis of Carbohydrate—Carbohydrates in the cell, how ever, appear in many forms, ranging from the simple monosacc harides such as glucose, glactose or fructose to the complex polysacc harides such as strach, glycogen, etc. The complex compound as such can not liberate the energy, and so the hydrolysis is brough about. The hydrolysis of starch and other polysaccharides, i catalyzed by amylase. The amylase present in the saliva produce by the mouth cells and also in the pancreatic juice, both of whic enhance the splitting of the polysaccharides to digosaccharides Ultimately, hydrolysis proceeds untill maltose, sucrose, and lactos are formed. *These disaccharides are then further hydrolysed t* monosaccharides under the influence of the enzymes maltase, sucras and lactase.

Hydrolysis of Lipid—The different lipids, which must be hydro lyzed, include the neutral fats, phosphatids, steroids, and the fa

soluble vitamins, A, D, E, and K. In the diet, the neutral fats are in greatest quantity. Neutral fats are hydrolysed under the influence of lipase to glycerol and fatty acids. It takes place as follows.

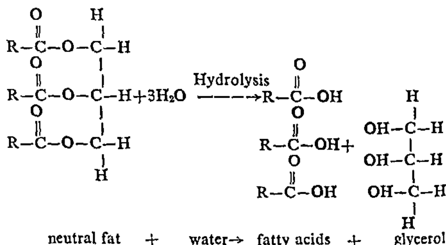


Fig. 14. Hydrolysis of neutral fat to fatty acids and glycerol.

It is worth mentionable here that surface tension between fat and water is very high. For this reason hydrolysis of fat, even in the presence of specific enzymes does not occur readily. The certain cells perform the function to produce bile which reduce this surface tension and also produce emulsification so that the fat droplet is broken up into very small drops of fats which present a much greater surface area than does a single relatively large mass.

Hydrolysis of Protein—In animals, there are specific enzymes in the gastric juice, in the pancreatic juice and in the intestinal juice which catalyze various stages of the protein hydrolysis. Pepsin in the gastric juice, however, initiates hydrolysis. This enzyme, if provided optimal conditions and sufficient time, is capable of carrying the hydrolysis all the way of amino acid stage, but in stomach polypeptide generally result. The rest protein and polypeptides are hydrolysed in the small intestine by trypsin. This hydrolysis of the different food stuff into their simpler component constitutes the phase I. The next step for further catabolic reaction is only possible after the hydrolysis is complete.

The next step to degrade the different end products of hydrolysis come under the phase II. These reactions mainly occur extensively within the cell.

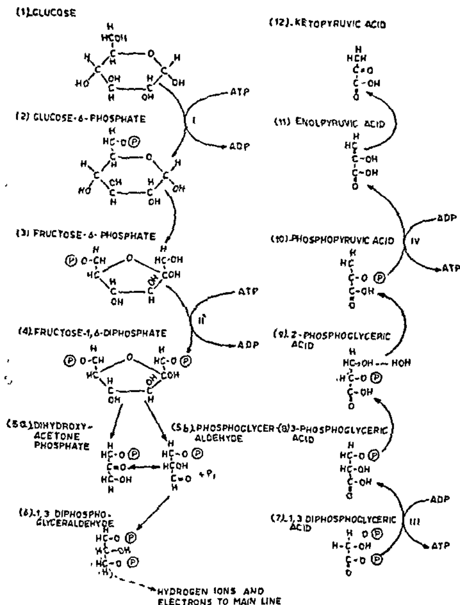


Fig. 15. Successive stepes in the anaerobic respiration (Glycolysis).

1. CARBOHYDRATE CATABOLIC PATHWAY

In most organisms energy is obtained primarily from respiration of carbohydrates and fats. Glucose is metabolized by several pathways but here we shall only discuss the one of the well established biochemical route which at present appears to be the

major respiratory pathway in plants, animals and numerous micro-organisms. The respiratory pathway consists of two phases, *i. e.*, **anaerobic respiration and aerobic respiration.**

The early sequence to the step in the respiration of most organisms, including man, is completely independent of oxygen and is called anaerobic phase of respiration. Many living things, including man, possess in addition to anaerobic respiration, a subsequent sequence of enzymatic reactions, which require molecular oxygen, is collectively called aerobic phase of respiration. In aerobic phase, the principal products of anaerobic pathway are further broken down to carbon-dioxide and water with the release of considerable energy. The anaerobic pathway of the process constitute the phase II of the reaction and the aerobic pathway contitute the phase III of the process.

1. **Anaerobic degradiation pathway of glucose or Glycolysis**— The term anaerobic respiration is often used inter-changeable with the terms **glycolysis** or **fermentation.** The fundamental biochemical step in the pathways can be summarized in the follow version and in the figure 15.

(1) The break down of the six-carbon sugar (glucose) into essentially two equal carbon molecules called glyceraldehyde.

(2) The oxidation of glyceraldehyde molecule to form glyceric acid and the removal of two hydrogen.

(3) The formation of pyruvic acid from glyceric acid by the removal of H and OH group.

4. In higher animal tissue the pyruvic acid is normally the major end product of anaerobic respiration for further process. If the oxygen is present then the pyruvic acid will go to the route of aerobic respiration.

5. In microorganisms, such as yeast, the pyruvic acid produce acetaldehyde under the anaerobic condition, which is than enzymatically reduced by accepting two hydrogens to yield ethyl alcohal.

Anaerobic respiratory pathway is in reality a process of brackdown, not of free glucose as such but of a phosphorylated derivative glucose. Thus the first steps in anaerobic metabolism therefore involve in conversion of glucose to its appropriate phosphate ester form.

Glucose phosphorylation—Glucose oxidation liberates a tremendous burst of energy with the formation of water and carbondioxide.

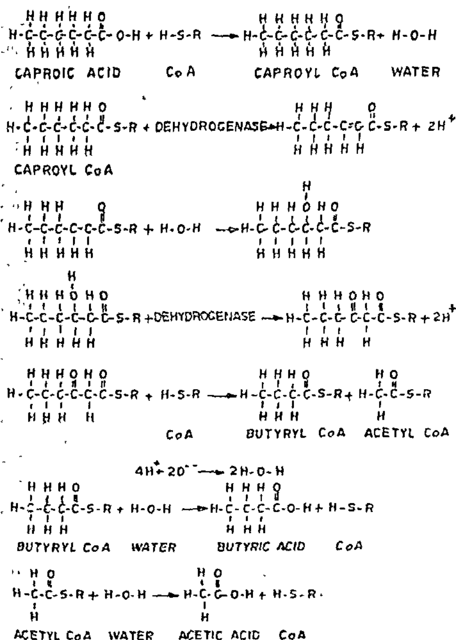


Fig. 16. Successive steps in the oxidation of fatty acid.

This occurs through a series of highly complicated interlocking steps. Adenosine triphosphate (ATP), a compound with high energy phosphate bond, generally enters into this reation as follows.

Hexokinase
Glucose + ATP————→Glucose six phosphate + ADP.

ADP stands for adenosine diphosphate, a substance which differs from ATP in that it has one less *high-energy phosphate bond.* This reaction of phosphorylation is catalyzed by the enzyme hexokinase. This type of enzyme is also known as glucokinase. Further glucose moves through the cell membrane more readily than does *glucose-6-phosphate.* Thus, the phosphorylation not *only* serve for the further metabolic process, but it also prevents it from *diffusing* out of the cell.

After phosphorylation isomerization occurs, which however form the fructose-phosphate from glucose-phosphate. The fructose-phosphate than again enters into the phosphorylation by ATP so as to form fructose-diphosphate and ADP.

(2) **Cleavage of phosphorylated fructose**—Fructose diphosphate is now enzymatically split between carbons three and four into two essentially similar phosphorylated three carbon molecules, glyceraldehyde-phosphate. This reaction corresponds to step one of the simplified version of anaerobic respiratory pathway.

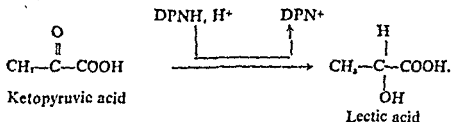
(3) **Oxidation of Glyceraldehyde-phosphate and formation of ATP**—Glyceraldehyde-phosphate is then enzymatically oxidised by donating two of its hydrogen atom to NAD and at the same time reacts *with* inorganic phosphate (P) to form NADH and diphosphoglyceric acid. The phosphorylating agent in this case is in organic phosphate drawn from the general mineral supply of the cell. The newly formed phosphate bond in the glyceric acid derivative is a high-energy type similar to that of ATP. In the presence of proper transferring enzyme the high energy phosphate is transferred from diphosphoglyceric acid to ADP to form ATP and phosphoglyceric acid.

(4) **Formation of Pyruvic acid and ATP**—The two subsequent enzymatic reactions of the structure of phosphoglyceric acid is altered and water removed to form phosphopyruvic acid. The high-energy phosphate of phosphopyruvic acid is now enzymatically transferred to ADP to form ATP and pyruvic acid.

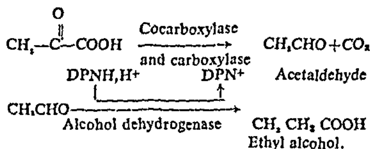
(5) **Fate of pyruvic acid**—Under normal circumstances the pyruvic acid formed by the anaerobic respiratory pathway would be further metabolized by the way of the aerobic respiratory pathway to carbon dioxide and water. In situation, in which molecular

oxygen is at time limiting, the pyruvic acids take the another route by the oxidation-reduction.

Under anaerobic condition, pyruvic acid act as a hydrogen accepter, forming lectic acid. In muscles cells, in oxygen debt may accumulate, a considerable amount of lectic acid as a consequence. The lectic acids is taken to the liver where part of it is oxidised to carbon-dioxide and water, and part of it is reduced to triose which form hexose phosphate. The latter is stored in the liver as glycogen. When the sugar is however needed to the body, the glycogen of the liver undergoes phosphorolysis and the sugar is released into the blood stream.



Muscle glycolysis—In the yeast cell another pathway is followed. The pyruvic acid is decarboxylated and the acetaldehyde formed is reduced to alcohol.



It should be kept in mind that neither the reduction of pyruvic acid to lectic acid nor the decarboxylation of pyruvic acid to acetaldehyde liberate energy. The heat of combustion of lectic acid is greater than that of pyruvic acid, as one might expect of a more highly reduced compound. Further the heat of combustion of acetaldehyde is the same as that of pyruvic acid.

A large number of the enzymes are involved in the reaction.

TABLE 1—ENZYMES INVOLVED IN GLYCOLYSIS PATHWAYS.

Reaction No.	Enzyme	Coenzyme
1—2	Glucokinase	ATP, Mg^{++}
2—3	Phosphoglucoiso-merase	—
3—4	6-phosphofructo-1-kinase	ATP, Mg^{++}
4—5	Fructoaldolase	—
5a—5b	Trioseisomerase	—
5b—6	Spontaneous	—
6—7	Glyceraldehyde-3-phosphate dehydro-genase	DPN^+, HOPO
7—8	3-phosphoglycerate-1-kinase	ADP, Mg^{++}
8—9	2-3 phosphoglyceric mutase	Mg^{++}
9—10	Enolase	Mg^{++}
10—11	Pyruvickinase	ADP, Mg^{++}
11—12	Spontaneous	—

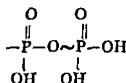
Further ATP, ADP system also performs another function, namely capturing useful energy released during respiration. ATP itself is exceptionally rich in chemical energy because it contains two "high energy" chemical bond as part of its chemical structure.

High energy phosphate bonds—It is evident from the foregoing [illegible]

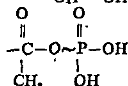
[illegible] of energy which they release on hydrolysis. One contain a low [illegible] the other a high energy phosphate bond ; the later is represented by the symbol ~. Low energy phosphate bond releases about 2,000 calories where as high energy phosphate bond releases about 12,000 calories, per mole, when hydrolysed.

Four types of high energy phosphate bonds are recognized.

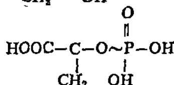
(1) To another phosphate group :



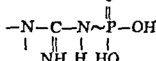
(2) To a corboxyl group :



(3) To an acidic enol group :



(4) To a *guanidine group :*



Example of the above types of the linkage are (1) ATP and ADP, (2) 1,3—diphosphoglyceric acid, (3) the enol of 2—phosphopyruvic acid, and (4) creatine phosphate.

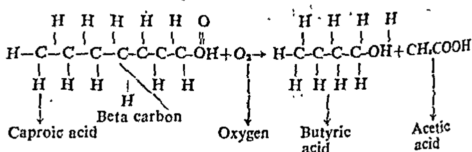
2—LIPID METABOLISM

Our knowledge about the lipid function and metabolism centers mostly about the fatty acids and the steroids. Fatty acids are present in the mammalian body largely in the form of triglycerides or fats. Within the cell itself, fats usually take the form of droplets in the cytoplasm. The main role of fats is to serve as a reservoir or store of chemical energy. They are usually in a considerably *more reduced chemical state than either carbohydrate and* proteins, possessing significantly more carbon-hydrogen bonds and, therefore, more chemical energy than either of these two classes of biological substances. Fats also function in the higher animals as a structural component of the living tissue.

Fatty acid oxidation—At last, the neutral fat break down into fatty acids and glycerol by enzymatic action. The glycerol is used *mostly for the synthesis of triglycerides and other lipid products.* The fatty acids at its goal of metabolism howevar, oxidised with the liberation of considerable energy. It would be recalled that fats yield 9·3 cal/3 when it is completely oxidized. There are many

theory about the fat oxidation, but the more accepted theory is "B" oxidation theory.

Oxidation of fatty acid, in this method, always occurs at the so-called beta carbon atom and as a result of this oxidation, a molecule of acetic acid is split off. However, a continued oxidation of the fatty acid shorten the molecule by two carbon atoms at each time untill the entire fatty acid molecule is oxidised.

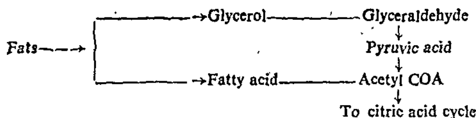


But it should be kept in mind that the oxidation of fatty acids is, in reality, far more complex. It is rather right that the long chain is split at the beta carbon atom, so that two carbon atom fragments come off, but several steps are involved in the complete process. Here it is clear that once again, starting with the six carbon compound, caproic acid, the first step is the union of CoA. Next, the oxidation occurs through the intervention of a dehydrogenase that however, causes the removal of two hydrogen ions. This intermediate product is then hydrated and is again oxidised by dehydrogenase with the loss of two hydrogen ions. Further, another molecule of coenzyme A plus an enzyme that however occur and causes the chain to breack, a so called cleavage enzyme enters into reaction. As a result of this, two esters of co-enzyme A are formed, one has four carbon atom and other has only two. The four hydrogen-ions that have been liberated by these reactions are accepted by oxygen, and thereby producing two molecules of water.

Further, the Acetyl coenzyme A can be converted by a molecule of water to acetic acid and coenzyme A, or it many go directly into the krebs cycle. Butyryl coenzyme A may react with the other molecule of water to become butyric acid and free the coenzyme A, or in the ester from it may be oxidised further, in another sequence, to result in two acetic acid end products.

N. B.—For more detail see author's book "Text Book of Animal Physiology" (*In Press*).

However, by this sequence of envents, long-chain fatty acids may be successively split by beta-carbon oxidation. The over all reaction follows as.



In certain cell, the presence of brown fat has been noted instead of white. Brown fat cells have many small fat droplets which are suspended in a cytoplasmic matrix. Brown fat has on oxidative power at least 20 times that of white fat. When animal cells containing brown fats are exposed to low temperature, these oxidative processes swing into action to produce remarkable quantities of heat. It has been suggested that the function of the brown fat is to produce heat as a protective measure against cold external environments. Further brown fat produces energy (heat) by means of oxidation of fatty acids. The brown fat cell first splits the fat in the droplets into glycerol and fatty acids. It then ejects the molecules of glycerol, but however, 90 percent of the fatty acid remain in the cell. The fatty acid then enters into the cycle

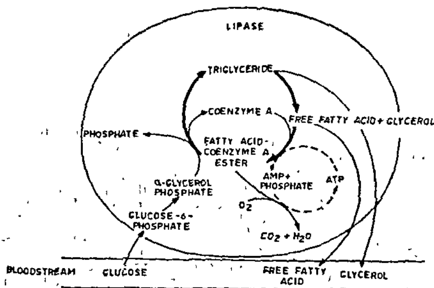
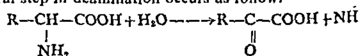


Fig. 17. Oxidation of fatty acid in Brown fat.

as shown in the fig. 17. It break down into their respective component and form triglyceride continuously so long as there is a continuing supply of oxygen and glucose. This cycle of catabolism and anabolism involves energy conversion, a part of which appears in the form of heat.

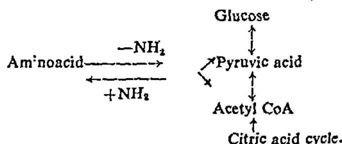
3—PROTEIN METABOLISM

In the cell, proteins may be enzymatically broken down or hydrolyzed into their individual amino acids and they may be further enzymatically degraded in several ways including removal of their amino group ($-NH_2$). This removal of the nitrogen or amino group from the carbon skeleton is, however, brought about either by *oxidative deamination* or *transamination* or by *transdeamination*. In the mammalian tissue primary first two methods are used for the purposes. Several specilised enzymes act in the process. The resulting organic products may be eventually metabolized to pyruvic acid and acetyl-CoA. Thus acetyl coenzyme A, a product of amino acid breakdown is also a link joining protein metabolism, carbohydrate metabolism, and fat metabolism to one another. The general step in deamination occurs as follow.



$$R-\underset{\underset{NH_2}{|}}{CH}-COOH + H_2O \longrightarrow R-\underset{\underset{O}{\|}}{C}-COOH + NH$$

OR



In the case of man these reactions occur mostly in the liver cell, but it also takes place to a limited extent in the kidney and intestinal mucosa.

Keto acid formation—The deamination of amino acids gives rise, ammonia and keto acid. The keto acids then undergoes various transformations. The amino acid leucine, tyrosine, and phenylalanine are converted to acetyl CoA : glutamic acid, histidine, proline and arginine to alpha-ketoglutaric acid ; and aspartic acid, tyrosine and phenyl-alanine to oxaloacetic acid.

Thus in this phase, the end products by the metabolic process are acetyl coenzyme A, alpha-ketoglutaric acid, and oxaloacetic acid. Three important products are now ready to enter into the final common metabolic path way.

Catabolism of nucleic acids—Nucleic acids can be broken down to their component parts through the action of several enzymes known in nucleus. Ribonuclease specifically hydrolyzes riboncleic acid, where as deoxyribonuclease hydrolyzes only deoxyribonucleic acid. They liberate polynucleotide and nucleotides may then be further hydrolyzed to yield inorganic phosphate and the base-sugar residue called a nucleoside. The latter is probably subsequently hydrolyzed to form the free base and sugar. The sugar may be eventually completely metabolised to carbon dioxide and water by way of one or more carbohydrate respiratory pathway. The purine and pyrimidine bases undergo different fats. In higher animals, the purine is not completely broken down but are excreted largely as a ring compound, with only small amounts, appearing as urea or ammonia. Pyrimidine in higher animals on the other hands are converted mainly to certain amino acids which may be excreted as such or used in other metabolic path ways.

2. **Aerobic catabolic path way**—The aerobic pathway consists of a succession of enzymatic reactions in which principal products of anaerobic respiration are ultimately oxidised to yield energy, water and carbon-dioxide. Although oxygen is a reactant in only the final step of the aerobic respiratory pathway, it is an indispensable reaction and the pathway would soon cease to function if oxygen is withheld.

The aerobic respiration can be divided into two main sequences of reaction known as (1) the **citric acid or kreb's cycle** and (2) the terminal respiratory, or **cytochrome pathway**. The first consists of a cyclic series of enzymatic reactions in which citric acid is one of several key intermediates. The terminal respiratory pathway in which several cytochrome participates involve the stepwise transfer of hydrogens or electron to oxygen from certain specific products of the citric acid cycle namely **NADH. NADPH**, and succinic acid, to form water. The formation of carbon dioxide in aerobic respiration occurs during the citric acid cycle sequence of event, where as the formation of most of the ATP take place in the terminal respiratory sequence. The fundamental biochemical changes occuring in the citric acid cycle using phyruvic acid as a starting material can be summarized in the following simplified version.

(1) **Oxidative decarboxylation of pyruvic acid**—This is actually a series of reactions involving the removal of carbon-dioxide and two

hydrogens from the pyruvic acid molecule. The resulting fragment of the pyruvic acid molecule then react with **coenzyme** A (CoA) to form an activated form of acitic acid called **acetyl** CoA. At least two enzymes and four coenzymes including NAD, participate in the reaction. The coenzyme A molecule, which includes in its structure the vitamin pantothenic acid, is present in general reserves of the cell.

(2) **Condensation of Acetyl-CoA and oxaloacetic acid**—The acetyl-CoA formed by the previous oxidative decarboxylation of pyruvic acid is now enzymatically condensed with oxaloacetic acid, which is present in the general reserves of the cell, to form the six carbon molecule, citric acid. CoA is liberated in the reaction and then returned to the general reserve of the cell.The citric acid is then enzymatically rearrenged to its isomer, isocitric acid.

(3) **Conversion of isocitric acid to ketoglutaric acid**—The isocitric acid now undergoes a transfer of two hydrogens to NADP to form NADPH followed by the removal of carbondioxide to produce the five carbon molecule ketoglutaric acid.

(4) ***Oxidative decarboxylation of ketoglutaric acid to succinic acid***—The conversion of ketoglutaric acid into succinic acid first involves the sequence of enzymatic reactions, *i. e.* analogous to the conversion of pyruvic acid to acetyl CoA. Two hydrogen and carbon dioxide are removed, and at least two enzymes and four different coenzymes, including NAD, which is reduced to NADH participate in the reactions. The succinyl-CoA that is formed is then enzymatically transformed in the presence of inorganic phosphate and ADP to succinic acid and ATP with the liberation of CoA.

(5) **Sucinic acid to oxaloacetic acid**—This is the final sequence of three enzymatic steps. In this the four carbon molecule, oxaloacetic acid, is regenerated, thus completing the citric acid cycle. It however, involve the removal of two hydrogens from succinic acid, and the addition of a molecule of water, and the transfer of two hydrogen to NAD to produce NADH.

The terminal catabolic pathway—The final stages of aerobic respiration deal with the enzymatic stepwise transfer of molecular oxygen of the electrons or hydrogens produced in the previous stages of respiration. These hydrogens or electrons are present as part of the structure of the NADH, NADPH and succinic acid. In the aerobic path way, NADH, formed during the oxidative

Fig. 18. Successive steps of aerobic respiration (kreb cycle).

decarboxylation of pyruvic acid to ketoglutaric acids and the eventual oxidation of succinic acid to oxaloacetic acid. **NADPH** is formed in the oxidation of isocitric acid. Succinic acid also makes available directly two hydrogens per molecule during its oxidation.

The union of hydrogen and oxygen so as to form the water is an energy-liberating reaction. In fact, when the reaction is performed in the test tube, it proves to be explosive. In the cell hydrogen is also united with oxygen but only after the hydrogen has first passed along a chain of molecules, the so-called electron transport system, during which small quantities of energy are liberated in a stepwise manner. An appreciable portion of this energy is however, captured through the formation of ATP from ADP.

The passage of hydrogen from NADH and succinate to oxygen proceeds by several enzymatic reactions of the terminal respiratory system through regular chain of the following cofectors the flavin coenzyme FAD, coenzyme Q and a reries of cytochromes designated as cytochrome b, c_1, c, a and a_3. The sequence of the components from NADH or succinic acid to oxygen is as follow.

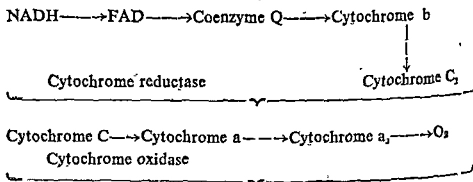


Fig. 19. Showing sequence of components of the terminal chain extending from NADH or succinic acid to O_2.

Each of the cofactor, during the passage of hydrogens through the terminal respiratory system, experience a reduction followed by a reoxidation as the hydrogen move on to the next components.

An important feature of the terminal respiration chain is its intimate association in mitochondria with a system for making ATP from ADP and inorganic phosphate. The process by which the energy is formed during the passage of electron through the terminal respiratory chain is known as **oxidative phosphorylation**. It is the principal mean for capturing the appreciable portion of energy liberated during respiration. For every molecule of NADH which is oxidised by oxygen through the terminal respiratory chain, three molecules of ATP will be formed from the ADP. The same relationship is suggested for the terminal respiration of NADPH. In the terminal oxidation of succinic acid, two molecules of ATP instead of three are formed per molecule of succinic acid oxidized.

ENERGETIC OF CATABOLIC PATHWAY

The primary role of this pathway is to provide available energy for the various functions and activities of the cell. The efficiency of anaerobic and aerobic pathway can be summarised as follows.

If the glucose as a typical substrate of respiration is completely burned and oxidised in furnace to carbondioxide and water (C_6

$H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O$), 686,000 calories per mole(180g glucose) are liberated almost entirely in the form of heat. Within the living cell the combined reaction of anaerobic and aerobic catabolic

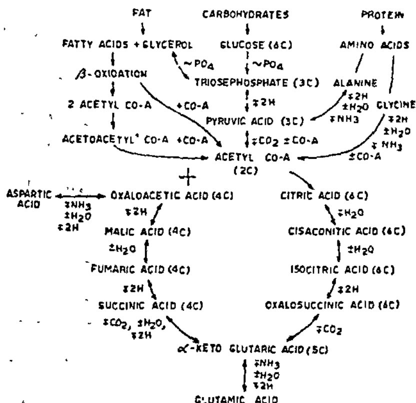


Fig. 20. Graphic representation of Glycolysis and Kreb cycle.

pathways accomplish the same complete combustion of glucose to carbondioxide and water with the same liberation of total energy. But the appreciable portion of this energy released, is captured in the bond energy of ATP, specially in the chemical bond between the second and third phosphate.

Thus in the aerobic respiratory pathway starting with the oxidation of pyruvic acid and proceeding successively through the citric acid cycle and terminal respiratory chain, a total of 36 molecules of ATP are produced (15 for each of the 2 molecules of pyruvic acid oxidised and 3 for each of the molecules of NADH arising in the aerobic pathway). This represents about 288,000 calories (36×8000 calories per moles of ATP) of the 626,000 calories originally present in the two moles of pyruvic acid and NADH which arose from the anaerobic breakdown of a single mole of

glucose. The efficiency of energy captured in the aerobic pathway is therefore about 46 percent (288,000/626200×100)=46 percent).

Further the oxidation of glucose by anaerobic pathway results in the release of less than 10 percent of the total chemical energy stored in the sugar molecule. Of the 686,000 calories per mole, only 60,000 calories are liberated by the anaerobic pathway. As a whole 4 ATP molecules are trapped from ADP and two are used in the process. Thus, the net yield of energy is only 2 APT instead of four with the resultant efficiency of 27 percent.

CENTRAL METABOLIC ROLE OF ACETYL—CoA

The central role of Acetyl-CoA is indicated by the fact that it is an important intermediate in several fundamental metabolic processes. As a product of carbohydrate metabolism, fat *metabolism*

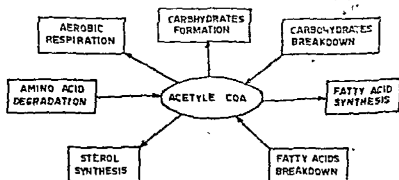


Fig. 21. Role of acetyle coenzyme A in linking carbohydrate, lipid and protein metabolism.

and aminoacid metabolism, it can be utilized in a number of different ways. Acetyl-CoA may be *completely broken down to* carbondioxide and water by means of the citric acid cycle and terminal respiratory chain thus serving as an energy source. It is also a building unit in fatty-acid synthesis, sterol synthesis, and carbohydrate synthesis. The important function of acetyl-CoA in linking carbohydrate, lipid and protein metabolism is shown in the figure 21.

FUNDAMENTAL ASPECT OF METABOLISM

The detailed biochemical reactions and mechanisms which constitute the various pathways and routes of metabolism appear to be overwhelming, a number of basic patterns bec[illegible] evident.

First, the respiratory processes are prima[illegible] of liberating chemical energy in the f[illegible] will [illegible] cell.

This necessitates that energy be released in small parcells, a phenomenon, *i. e.* attain in the nature by numerous enzymatic reactions instead of a single large energy-yielding reaction.

Secondly, the cell capture the useful energy in the form of ATP which serves as a direct source of energy for all life activities. ATP is the universal biological substance which is immediately involved in the energetics of all major routes of metabolism of carbohydrates, fats, proteins and nucleic acids, as well as the other energy requiring activities.

Thirdly, certain substances, specially coenzymes are widely distributed in biological system participating in numerous pathways of metabolism. The coenzyme NADP and NAD, serve as hydrogen or electron carriers in anaerobic pathway, aerobic pathway and fat metabolism.

Fourth, in a number of biochemical reactions, the substances or reactants must be in a so called "activated" state. This is frequently achieved by forming a derivative with coenzyme A in a reaction that require an energy input which is usually provided by ATP.

Fifth, the various pathways of metabolism within the living cell are in one or more ways interlinked with each other as through acetyl-CoA, which form the intermediate key of the reaction.

Finally, the entire complement of enzymatic reactions making up the metabolism of the cell is under the basic direction and control of DNA in the nucleus. The vast quantity of information enclosed in the molecular structure of DNA is ultimately expressed in the protien (*i. e.* energy), the fundamental machinery of all living cells.

ASSOCIATION OF METABOLIC PATHWAYS WITH CELL STRUCTURE

The elucidation of the various routes of metabolism has been inevitable corrected with the distribution and localization of these pathways among the subcellular components themselves. These can be summarized as follows :

In many tissues of the cells, the enzymes of anaerobic pathways are located in the cytoplasmic matrix other than the mitochondria and endoplasmic reticulum. The aerobic pathway, however, appears to reside almost completely in the mitochondria. The enzymes of the citric-acid cycle are relatively lightly bonded and organized with the mitochondria. The terminal respiratory chain is part of the

membranous structure (cristae of the membranes) of the mitochondria, and fatty acid synthesis occurs mainly in the endoplasmic reticulum. Protein synthesis, as our knowledge is at present, take place largely on the ribosomes. With respect to the nucleic-acid metabolism, DNA is synthesized in the nucleus and RNA is synthesized in both the nucleus and the cytoplasm. Degradiation of the different products probably occurs at the similar localities.

SUMMARY

The aggregate of all the physical and chemical processes constantly taking place in the living cell is termed as metabolism. These processes include those reactions which utilize energy to synthesize complex substances from similar elements as well as the reactions which release energy. The processes which result in biosynthesis are embraced by the term anabolism, while catabolism includes all reactions in which complex molecules are converted to smaller ones, generally with the release of energy.

Biosynthesis is the formation of complex molecules from simpler ones by the cell. Plant cells are capable of photosynthesis, a process which uses the radiant energy of light for the formation of carbohydrate from CO_2 and H_2O. The monosaccharides so formed can be converted by the intracellular processes to oligosaccharides and also to glycogen. Fatty acids can be conjugated with glycerol to form neutral fats. They can also be combined with phospholipids. Another product of lipid biosynthesis is cholesterol. Aminoacids are used by the cell for the synthesis of polypeptides and protein.

Many organisms can give rise to bioluminescence. This serves for sexual attraction, protection, and attraction of prey. The colour of the light is generally in the orange-yellow-green-blue range of the spectrum. Luminescence involves the oxidation of luciferin catalyzed by luciferase. Oxygen and ATP are essential for this reaction and may be involved in the nervous control of luminescence.

The reactions which liberate energy is said to be exergonic. The hydrolysis of the basic food stuffs during digestion involves exergonic reactions. The end products of carbohydrates digestion are the monosaccharides of lipid digestion, glycerol and fatty acids ; and of protein

digestion, the aminoacids. This includes the first catabolic pathway of the exergonic reactions.

The second catabolic pathway includes, the degradation of monosaccharides, fatty acids, glycerol, and some of the aminoacids into acetyl coenzyme A. Other aminoacids form alpha-ketoglutaric acid and oxaloacetic acid.

The third phase of the catabolic reactions include the tricarboxylic acid cycle. This is the cycle of interrelated or coupled reactions. However, all of the constituents which take part in the cycle are regenerated with the exception of acetyl CoA. So long as the oxygenis available and acetyl CoA is abundant, the cycle will continue to operate indefinitely with the production of H_2O and CO_2 and with the liberation of considerable energy. For these oxidation-reduction interactions many specific enzymes are required. These includes the oxidases and dehydragenases. The cytochromes are important oxidases. The flavoproteins are dehydragenases which function in the conjugation with coenzyme I, nicotinomide-adenine-dinucleotide (NAD) and coenzyme II, nicotinamide-adenine-dinucleotide phosphate (NADP). The energy released during the tricarboxylic acid cycle is generally used for the transformation of ADP to ATP.

At last the role of acetyl CoA has been dealt in the metabolic pathways. This product however act as an intermediate substance of the carbohydrate, fat and protein metabolism. It has been noted that various routes of metabolism have been inevitably correlated with the distribution and localization of these pathways among the subcellular components themselves.

4

PROTOPLASM

Although there is a continuous stream of matter and energy, flowing through the living individual, nevertheless the physical and chemical study of living matter from whatsoever source we take reveals a striking similarity in its fundamental factor, this is the basis of the protoplasm concept held by modern zoologists.

The term protoplasm (Gr : *Protos*=first+*plasma*=substance) has been variously defined as "living matter," "living substance", and "the physical basis of life". But to treat a living unit as alive is not correct, for only the complete life unit is alive. None of its part is of itself alive. It is equally a mistake to use the term protoplasm in any sense implying that there is but one substance common to all kinds of living cells. The structure of cell vary considerably in different kinds of cells and yet the active materials of all of them equally deserve to be called protoplasm. Thus we can simply say that protoplasm is only a convenient substitute for, "the complex colloidal system constituting the active part of the cells' with all its proteins, lipids, carbohydrates, enzymes, salts and water." Their proportions and interrelations are variable not only from organism to organism but also from cell to cell. Even in the same cell, they can show variations from time to time. In short, the protoplasm is a highly organized and has a dynamic nature.

Protoplasm is a vastly complex arrangement of the ions, molecules and colloidal particles. It is uniquely organized into a multiplicity of structural and functional systems which all collectively display the characteristics, what is known as living system. The different constituents are organized for the most part into a variety of units which again combined together to form a large and more intricate units or systems (e. g. mitochond [illegible], etc.)

the first time, the presence of gummy and soft substance from the animal tissue. He named it sarcode (=a substance taken out from the flesh). In 1840 **Purkinje** gave the name protoplasm to this substance. Great German Botanist **Hugo von Mohl** in 1846 also suggested the name protoplasm to a granular and viscous substance found in plants and animals:

Microscopic Structure of Protoplasm—Different scientists have attributed the different forms to the protoplasm which led to various theories for the finer morphologic structure of the protoplasm. However, in the light of modern knowledge the following theories have been given.

1. **The Granular Theory**—**Altamann** (1893) stated that the protoplasm is made up of tiny units or granules of varying sizes that are sometimes massed into solids and sometimes arranged in linear series so as to form fibrils. Some scientists called these granules as **bioplasts** due to their living activities.

2. **Fibrillar Theory**—It was suggested by **Flemming** who stated that the protoplasm consists of reticulum or network of fibres. These fibres are embedded in matrix.

3. **Reticular Theory**—It emphasizes the fibrous make-up, and adds the conception that the fibres are knotted together into an intimate network. This nature of protoplasm is well evident in the nerve cells.

4. **Alveolar Theory**—This theory was suggested by **Butschli** (189?) [illegible] oplasm is a sort of foamy emulsio [illegible] mixed; these are the ground sub[illegible] ure with spheres of more liquid material suspended in it. Protoplasm appears to be comprisng large number of alveoli or minute bubbles in a comparatively viscid liquid medium.

5. **Colloidal Theory**—**E. B. Wilson Fisher** (1894) has stated that the protoplasm is a colloidal solution. **Wilson** further stated that the so called "alveolar structure is not a primary characteristic of the protoplasm. It is of secondary origin, arising by the appearance in the homogeneous ground substance of extremely minute scattered [illegible] g together finally prod[illegible] Wilson concluded "tha[illegible] structure can be given......It is impossible to resist the evidences that fibrilar and granular as well as alveolar structures are of wide occurence; and

while each may be characteristic of certain kind of cells or of certain physiological conditions, non is common to all forms of protoplasm." This can be said only at present, that the protoplasm is a complex mixture of substances which may assume various forms of visible structure according to its mode of activity.

CHEMICAL COMPOSITION OF PROTOPLASM

Elements—In all, about 34 elements have been detected in the protoplasm, out of which about 12 are universally present ; these are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, chlorine, sodium, potassium, calcium, magnesium, iron and iodine. It is striking to note that 96 percent of the whole body is composed of only four very common elements, *i. e.* oxygen, carbon, hydrogen, and nitrogen, in their various combinations. Weight has given the following percentage of different elements, apart from the intercellular substance.

Oxygen	(O) ...	76%	Potassium	(K) ...	0·3%
Carbon	(C) ...	10·5%	Iron	(Fe) ...	0·01%
Hydrogen	(H) ...	10·0%	Magnesium	(Mg) ...	0 02%
Nitrogen	(N) ...	2·5%	Calcium	(Ca) ...	0 02%
Sulphur	(S) ...	0 2%	Sodium	(Na) ...	0 05%
Phosphorus	(P) ...	0·3%	Chlorine	(Cl) ...	0·10%

With these elements, the traces of silicon, copper, aluminium, maganese, boron, carbondioxide, iodine, flourine, and bromine are also present in the protoplasm.

Compounds—Elements do not occur independently in the protoplasm but they combine to form either the organic compounds or inorganic compounds. The organic compounds form more than 35% of the whole of the protoplasm and comprise mainly the proteins, the lipids, the carbohydrates, and the enzymes. With these organic compounds, the water, gases and certain inorganic salts are also present.

Water—water is the main camponent of protoplasm and occurs in large amount. It serves as a natural solvent for other materials and olso plays a major role in the metabolic activities, since physiologic processes occur exclusively in an aqueous medium. The water molecules also participate in many enzymatic reactions in the cell. No doubt, certain amount of water is formed in the cell as a result of metabolic processes, but this is unsufficient to ...tain the water balance of the body. Therefore, it must be and

is supplied from the outside. Inside the cell, the water exists free *i. e.* a solvent as well as bound, *i. e.* tied to polar groups of protein molecules by hydrogen bonds. Inorganic compounds always occur as charged ions in the protoplasm. They may be positively charged particles or cations such as Na^+ or negatively charged particles or anions as Cl^- in equilibrium.



$$NaCl \rightleftharpoons Na^+ + Cl^-$$

The distribution of the ions of various salts inside and outside the cell is important in maintaining the osmotic balance in the cell. Certain ions are of particular importance to the organisation of protoplasm and its metabolic activities. One of the most important group is the phosphate group (PO_4^+) which is associated with phosphoprotein, phospholipid and nucleotide. Excess of any one salt is injurious to protoplasm. The different ions have a sort of regulative action one upon the other so that the toxic effect of any given ion is neutralized.

Sulphur is another important inorganic constituent in the organisation of several organic compounds, such as aminoacid. Such inorganic ions as manganese and magnesium may serve as cofactor for the activity of specific enzymes. Norman Cohn (1964) gave the following composition of different compounds in the protoplasm.

TABLE 2—SHOWING DIFFERENT COMPOUNDS OF PROTOPLASM

Substances	Relative Frequency	Location in cell
Water	0·09±0·02	Nuclear sap, vacuoles, hyaloplasm.
Proteins	0·70±0·02	Hyaloplasm, enzymes, membrane.
Carbohydrates	0·02±0·005	Vacuoles, hyaloplasm, cell wall, storage products.
Lipids	0·01±0·005	Membranes, hyaloplasm, inclusions.
Inorganic materials	0·01±0 005	Vacuoles, hyaloplasm, cofactors.

1. Proteins and Aminoacids—Proteins are the framework of

protoplasm. It gives to the protoplasm a complex structure and properties. They differ in their composition from carbohydrates and lipids in that, in addition to carbon, hydrogen, and oxygen, they always contain nitrogen and sometimes sulphur and phosphorus. It is usually present in different forms in different parts of cell. In hyaloplasm, the proteins are usually present in the form of principal ingredient of the protoplasmic gel. From cytologic point of view, it can be said that there exists both simple and conjugated proteins. The simple proteins are compounds which on hydrolysis yield exclusively alpha-aminoacids. The important simple proteins include the **albumins** which are soluble in water and are coagulable by heat ; the **globulins** which are insoluble in water but soluble in dilute salt solutions ; the **protamines** which are strongly alkaline component of the sperm cell ; and the **histones**. The histones are less basic and are found in many cell nuclei. The conjugated proteins are those where other substances of organic nature, called the prosthetic group, combine with the simple proteins. They on hydrolysis yield alpha-aminoacids and an organic component. The important conjugated proteins are the **nucleoproteins**, **glycoproteins**, the **lipoproteins** ; the **chromoproteins** comprising haemoglobin, haemocyanin, and respiratory enzymes such as cytochromes, flavoproteins, etc.

Proteins are composed of aminoacids or they are the polymers of aminoacids. Aminoacids are amphoteric compounds because they contain both acid and base groups. Proteins are usually regarded as polypeptide chain (a polypeptide is a compound of several aminoacids), as such they have high molecular weight. The characteristic arrangement of carbon, hydrogen, oxygen, and nitrogen atoms in the aminoacid is typical in the form of amino ($-NH_2$) and carboxyle ($-COOH$) groups as given below.

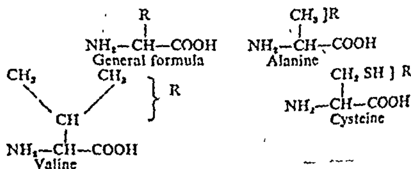


Fig. 22. Different Aminoacids.

In the Fig. 22 R—group represents the particular specificity to the aminoacid, *i. e.* that differentiate one aminoacid from the other.

A protein consists of a chain of these aminoacids. The number, order and kind of aminoacids, however, determine the type of protein activity in the cell. The chain organisation arises from the amino and carboxyle groups. As the amino group is basic and carboxyle group is acidic, the formation of the chain depends upon the combination of the amino group of one aminoacid with the carboxyle group of another. A molecule of water is lost in the process.



$$R\,COOH + RNH_2 \rightarrow R—CO—NH—R + H_2O.$$

The linkage—CO—NH—is known as peptide bond. Such a chain formed by the combination of two aminoacids is called a **dipeptide**; where there are a few aminoacids, the chain is called an **oligopeptide**; whereas the chain which arises by the combination of many aminoacids is a **polypeptide**. Emil Fischer (1902) suggested that the aminoacids are arranged in the following manner by polypeptide bond.

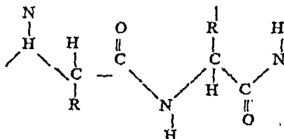


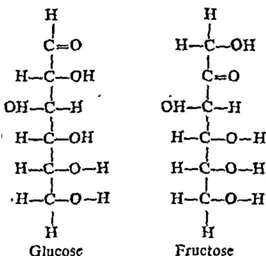
Fig. 23. Protein molecule.

Moreover, the arrangement of aminoacid in protein chain, is not always linear but may contain, one or more turn. There are over 20 aminoacids and many more proteins of biological importance, some are listed on the next page.

Proteins are generally large molecules with high molecular weight ranging from about 12,000 to over 2,000,000. Several proteins also act as enzymes. The formula of ribonuclease, the first enzyme or protein whose structure was determined, consists of several turns in a chain of nineteen different peptides with a molecular weight of 14,000. Some enzymes require a non-proteinous addition for the activity. If this addition is of organic nature, it, is usually called prosthatic group and if it is of inorganic nature, it is commonly called as cofactor. In a cell, the enzymes are found associated with several cellular components like endoplasmic reticulum, plastids, mitochondria, etc.

divided into three classes, *i. e.* monosaccharides, disaccharides, and polysaccharides. The first two are commonly called as sugars and are readily soluble in water. They can be crystallized and can easily pass across dialyzing membranes. The polysaccharides form colloidal solutions with water, do not crystallize, and do not pass membranes.

The monosaccharides are simple sugars with an emperical formula $C_n (H_2O)_n$. They can be classified as trioses, pentoses, hexoses, and heptoses, in accordance with the number of carbon atoms. The most important monosaccharides in the cells comprise pentose and hexoses. These two are of great biological importance. Ribose and deoxyribose are the pentose sugars forming components of nucleic acid molecules. Hexoses are important for food and nutrition. The important hexoses are glucose and fructose. The glucose plays an important role in the metabolism. It is soluble in water and occurs in the cell in all the vertebrate and invertebrates. All carbohydrates taken in food are broken ultimately into glucose in the body, which is then absorbed by the blood and then transported to the cell. The amount of glucose in human blood is 100 mg. per 100 ml. If its concentration increases, diabetes results. The fructose (fruit sugar) has the composition similar to glucose but differ in the chemical properties and in physiological role. This is due to the differences in the position of the—OH group in the two molecules as shown below :

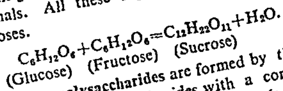


Glucose Fructose

They are the isomer to one another

The disaccharides are sugars formed by the condensation of

two molecules of monosaccharides with the loss of one molecule of water with an emperical formula $C_{12}H_{22}O_{11}$. The main substances of this group are sucrose, and maltose in plants, and lactose in animals. All these sugars are derived from the condensation of hexoses.



$$C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O.$$

(Glucose) (Fructose) (Sucrose)

The polysaccharides are formed by the condensation of many molecules of monosaccharides with a corresponding loss of water molecules. Their emperical formula is $(C_6H_{10}O_5)_n$. They yield molecules of simple sugars on hydrolysis. Biologically the important polysaccharides are the starch, the glycogen, and the cellulose. The former two are the reserve substances in cells of plants and animals respectively where as the cellulose is the characteristic structural element of the plant cells. The **starch** $(C_6H_{10}O_5)_n$ is a mixture of two long polymer molecules, *i. e.* liner **amylose** and branched **amylopectin.** It has no structural importance in animals. The glycogen $(C_6H_{12}O_6)_n$ is generally called as the starch of animal cells. It is a polymer composed of many molecules of glucose and thus represents an important reserve of energy in the body. Though found in numerous tissues and organs, but contained in liver cells and muscle fibres in greatest proportions. It forms a colloidal solution in the protoplasm. The **cellulose** is composed of hundred of monosaccharides It has no structural importance in animals except the tunicates which have a wall of cellulose. It takes part in the formation of cell wall and also of a series of other structures which form the supporting skeleton of plants.

Besides cellulose, plant tissue contain several other structural components such as xylam, alginic acids (in algae), pectic acid, etc.

Thus we can conclude that carbohydrates play three main roles in the cells. These are :

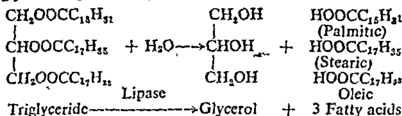
1. They furnish the most readily available fuel.
2. They are important articles of food storage.
3. They have a minor role in furnishing part of the structural material or atleast one part of the essential environment of livin cells.

3. **Lipids**—The lipids are structural components of the cyt plasmic membrane and also appear as insoluble inclusions in t

hyaloplasm. They are fonnd abundantly in the form of droplets scattered through the protoplasm, acting as reserve food packages. Phospholipid is an important lipid compound present in the various cytoplasmic organelles. They are also made up of carbon, hydrogen, and oxygen but the ratio of hydrogen, and oxygen is much greater than 2 : 1.

Lipids can be classified as below :

1. **Simple lipid**—These are alcohol esters of *fatty acids*. They include (a) **Natural fats** or **Glycerides** or often called triglycerides. These are the triesters of fatty acid and glycerol. They are further divided into fats and oils. Common fats are tallow, lard, human fat where as the common oils are fish oils, caster oil, olive oil, etc. (b) Waxes—These are the esters of fatty acid with alcohol other than glycerol *e. g.* Beewax,



2. **Steroids**—These are the complex lipids such as sex and adrenal hormones, vitamin D, the bile acid, etc. Steroids with an OH group are called **sterols** such as cholesterol which is the principal constituent of wool fat and is also found in the bile, brain, adrenal glands and other organs.

3. **Conjugated lipids**—They on hydrolysis yield other compounds in addition to alcohol and acids. They comprise phospholipid, cerebrosides, carotenoides.

Enzymes—Possibly no more important substances exist in the protoplasm than the enzymes. They are found not only in cells but are secreted by cells into the blood and into the digestive tract, where they act as **catalizers**, *facilitating* various special chemical changes. Each enzyme is specific for a particular kind of chemical reaction. They are conjugated proteins, formed by the cells from aminoacids with the help of peptide chains. A single cell has thousand of enzymes to accelerate its hundredth of chemical and physiological reactions. Their activities are controlled by many factors, the most important are the temperature and the pH. The optimum temperature at which the reaction takes place rapidly for a particular enzyme is normally around 37°C to 45°C. In the cell, the

found in association with several structures like mitochondria, plastids, nucleus, and the endoplasmic reticulum. The cell enzymes can be classified on the basis of their reactions in hydrolases, oxidases and desmolases. Hydrolases break up the complex molecules into simpler ones by the addition of water while oxidases are connected with biological oxidation and reduction. Desmolases catalyze the rupture of linkage which are not hydrolyzable.

Nucleic acid—The nucleic acids are specially significant substance, found in the nucleus and the cytoplasm. In the nucleus, they are associated with the chromosomes and are important in transmitting the information from the nucleus to the cytoplasm. In the cytoplasm, they are most closely concerned with the synthesis of protein. The nucleic acid consists of a ribose or deoxyribose sugar, nitrogenous bases (purine and pyrimidines), and phosphate. The two biologically important nucleic acids are ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The details of these two types of acids has been discussed in separate chapter, given ahead. The nucleic acid is actually composed of chain of nucleotide units. A nucleotide unit consists of a molecule of sugar, a base and phosphoric acid. The single nucleic acid contains large number of nucleotide units and has a high molecular weight.

Physical properties of protoplasm—From the physio-chemical point of view the protoplasm is regarded as a complex colloidal system. As a result it has primarily the characteristics and properties of macromolecules in solution. The term 'colloid' is applied to a system which involves particles or aggregates ranging in size from about 1 mμ to 500 mμ. Now-a-days the colloidal particles are measured in terms of number of atoms rather than millimicrons. These particles or aggregates may show some of the properties of solutions, sometimes they behave like suspensions, and may bear an electric change and migrate in an electric field. Hence they behave like ions.

In a colloidal system particles or aggregates constitute the so called dispersed phase which is suspended in the dispersion medium. For example in colloidal gold, the gold particles are the dispersed phase and the water is the dispersion medium.

Dispersed phase + Dispersion medium = Colloidal solution.

As already stated, water is the dispersing phase of the protoplasmic colloid. In this glucose, inorganic salts, protein and glycero-

particles occur in dispersed phase. Different fats occur in the form of fine globules. Due to this admixture of both solid particles and liquid globules in the dispersed phase, protoplasm is said to be a compound colloid. Recent studies with polarized microscopes show that on the periphery of cell like *Amoeba*, there exists a hyaline zone of clear protoplasm. Such condition is known as **isotropic** while the granular protoplasm met with inside is called **anisotropic** condition. Anisotropic material shows the double refraction of light when it passes through the crystal of calcite ; light is then *deviated resolved into a deviated extraordinary ray. This property* of protoplasm, showing double refraction is known as birefringence.

Some of the properties of the colloidal system and so the protoplasm are as below :

1. **Brownian movement**—With the help of microscope, minute granules or particles have been observed moving in protoplasm. This movement of colloidal particles was first observed by an English botanist **Robert Brown** in 1828 who discovered the nucleus. The movement is named after *him as* ***Brownian movement****. It is proposed* that the movement is due to the collisions of the molecules of the dispersion medium against the colloidal particles. Brownian movement depends on the size of the particles and the viscosity of the medium and is proportional to the temperature. If the temperature of the liquid is higher, the thermal agitation of molecules will be more rapid with the result the bombardment of the particles will be more frequent.

2. **Tyndall effect**—When a beam of light passes through a colliodal solution, the path of the light becomes visible which is produced by the scattering of light from the surface of the colloidal solution and it is termed as Tyndall cone which appears to have a faint blue cast. This phenomenon is called "Tyndall effect" (T.E.) This phenomenon is the basis of the ultramicroscope or dark field microscope through which one can observe the location of colloidal particles by noting the position of the points of light which they scatter. Protein colloids generally show no marked T. E. in visible light, although *they scatter ultraviolet light.*

3. **Gel**—The macromolecules are characteristic in having the capacity to form gels, *i. e.* solutions which show mechanical pro-

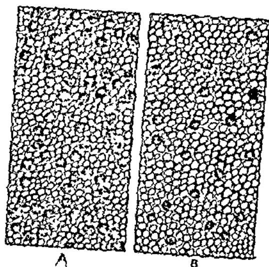


Fig. 24. Change of sol into gel (A) sol ; (B) gel

perties comprising viscosity, elasticity, *tensile strength*, etc. At lower temperature the gel is converted into sol. For example, if gelatin is dissolved in hot water it forms a liquid solution (sol.) This solution becomes viscous and elastic *gel if the sol. is allowed* to cool. Gels are often *liquified* (sol.)by changes in temperature, pH, salt concentration, pressure, etc. This change is called the *solation*. *This sometimes* be reversed by removing the factor involved *with the result gelation* occurs. Gel has the property of *contractility*. On contraction, gel expels some liquid along with the *dissolved substances in the latter*. *This* common process is called *syneresis*, *i. e.* extrusion of serum from clotted blood.

4. Protoplasm has the property of absorbing and eliminating water. This might be one of the reasons for its continuous changes *in viscocity*. *This* property has great significance in the vital *process*. Thus in muscular contraction, there is probably a change *in water* distribution inside *the fibre*. In all probability, the motion of many *plant calls is due* to the similar cause.

The viscosity can be defined as, "a manifestation of molecular attraction". *In true sene* it is always inverse of fluidity. A highly *viscous substance* is one that offers great resistance to a change in form. This property is derived from the molecular attraction between *the different molecules of the* substance. Protoplasm may be considered to be highly *viscous liquid* at least in *comparison with water*. The viscosity of the protoplasm from the different *cells have been measured*. *They however*, vary in different cell, but in general *they seem to be in* the range of 2 to 20 centipoises. *(viscosity in* comparison of the water whose viscosity value is 1 centipoise).

5. Colloids tend to form a membrane at the surface as a result plasma membrane surrounds a cell. It is formed of closely

packed colloidal particles of proteins and lipids in the gel state. This membrane determines what shall pass into or out of the protoplasm.

6. **Osmosis and Osmotic pressure**—One of the most significant physical phenomenon associated with colloidal membranes is osmosis, and the pressure exerted thereby is called the osmotic pressure which may be very powerful. It plays a very important rôle in the life processes. Many important phenomena in cells depend upon this semipermeability of their membranes. Becuse of this, the cells are kept plump and well rounded because the osmotic pressure on the inside and on the outside of the plasma membrane is equal.

7. **Surface precipitation reaction**—**Heilbrunn** found that protoplasm has the capacity of surface precipitation reaction i. e. if a cell membrane is formed artificially, the protoplasm will flow through it and soon forms an enveloping membrane around it. For the activity of precipitation reaction calcium is essential element.

8. **Formation of vacuoles**—Protoplasm has the tendency to form vacuoles when the cell is ruptured or exposed to cold or heat or to ultraviolet radiation. This tendency is closely associated with surface precipitation reaction. **Heilbrunn** pointed out that it is due to the release of calcium in the protoplasm of the injured cells.

9. **Hydrogen-ion-concentration**—The aciditiy or alkalinity of protoplasm indicates its colloidal nature. In true sense, the protoplasm is a colloidal solution of
which ionises into acids as well
is variable and seems to effect its activity greatly. Chambers believed that the cytoplasmic pH varies from 6·8 to 6·9 whereas Spek held that the pH of cell shows a wide range, probably between 5 to 8 and can vary even in different parts of the cell.

10. **Inherent structural bonds**—As shown before protoplasm has a reticular appearance. It has been noted by experimental observations that protoplasm is a structure with elastic properties only in some areas, usually the cortical layers. It has also been noted that there are probably structural bonds of some nature in protoplasm. It is shown by the experiments, that when homogenous fluid exhibits a straight line relationship between force and flow, a very different
considerable force is needed
line is not straight at least a

been explained on the basis of structural bonds in protoplasm which oppose flow so that it only occurs when sufficient force is applied to break the bonds.

11. **Light refraction**—The ratio between the velocity of light in a substance compared with that in air, or a vacuum, is termed as refractive index. Thus the refractive index for air is 1·0, for protoplasm it is about 1·4. Such measurements provide the view, that protoplasm is homogenous, which it is not. Certain parts of the cell exhibit, double refraction, or birefringence. Protoplasm itself does not usually exhibit double refraction, but various structure located in the protoplasm often do so. These include the nuclear membranes, the chromosomes, and the mitochondria. The most significant point of investigating the refraction of light as it provides the valuable information as to the orientation of their constituent molecule. Further, the orientation of each molecule is suggested by the manner in which light is refracted.

Biological properties of Protoplasm—It comprises all the vital activities going on in the living body ; the activities which differentiate the living being from non-living beings. These activities may be referred as metabolism, nutrition, growth, excretion, respiration, irritability and reproduction.

1. **Metabolism**—It is the sum total of different biochemical activities which however occur in the protoplasm. The life cannot go on, if metabolism ceases, for the ceasation of metabolism the organised unity of cells breaks down and even the colloidal character of the material becomes disorganised.

2. **Nutrition**—Every living organism requires some kind of energy which is always gained through the nutrition. Food is used by the organism which is then digested and assimilated for the good.

3. **Respiration**—The energy is produced by the oxidation of the protoplasm and the different food with the result waste CO_2 is produced. No animal and plant can live without the fresh supply of oxygen. So the exchange of gases is very necessary for the protoplasm.

4. **Excretion**—Protoplasm has the capacity to excrete the wastes such as urea, uric acid, CO_2 and many others which are produced during different metabolic activities.

5. **Growth**—It is also a biological activity of protoplasm. As a result of different metabolic activities (specially anabolic) growth

occurs in the living body. The method of growth differs from that of non-living units, such crystals in that it takes place through **Intussusception,** *i. e.* by the increase of every minute part of the active material.

6. **Irritability**—Protoplasm has the high capacity of irritability through the process of stimulation. This stimulation may be due to the physical factors like heat, light and gravity or due to the chemical reactions.

7. **Reproduction**—Due to the different metabolic activities and growth in the animal body, reproduction has become the essential phenomenon of living life. By this process the animals and plants produce their own image so as to continue their progeny.

8. **Conductivity**—The protoplasm has the capacity to conduct the impulses produced by stimuli from one part of protoplasm to another.

SUMMARY

Protoplasm is a vastly complex arrangement of ions, molecules and colloidal particles, uniquely organized into a multiplicity of structural and functional system which collectively display the characteristic of living system. It exhibits so many forms. The intracellular pH have been noted which range between 5·8 to 8·5. However, in the living state the pH range probably very close to neutral, *i. e.* 7. Chemically protoplasm is made up of 85% water, 10% protein, 2% lipid, 1·5% carbohydrate and 1·5% salt. **Proteins** are made up of different amino acids, that unite to form peptides, than peptones and finally proteoses. At last it form a large size of protein molecule. Due to the size of protein molecules, the diffusion is slow and most membranes will not permit them to pass. Amino acids are termed or **dipolar ions,** because they have both negative and positive charges. However, the relationship of these charges in the protein molecule determines its isoelectric pH. Proteins combine with nucleic acid to form **nucleoproteins** ; they also unit with lipid and various ions. The characteristic of protein is determined by its amino acid sequence. There are 20 amino acids,

Carbohydrate are composed of carbon, hydrogen and oxygen. The most simplest carbohydrate are the monosaccharides which combine to form dissacharides and polysaccharides. The pentose monosaccharides are of particular importance to protoplasm because they appear in ribonucleic acid, and deoxyribonucleic acid.

Lipids comprises, neutural fats, phosphatids, waxes, steroids other substances. Neutral fats are composed of three molecules of fatty acid and one of glycerol. The more important steroids are cholesterol, ergosterol, and many hormones.

Protoplasm is a viscous liquid. The unit of viscosity are the poise and stoke. The viscosity of the protoplasm varies from cell to cell in the range of 2 to 20 centipoises in comparison to water. Protoplasm have a definite structural bonds that resist the movement of object through it.

Protoplasm possesses all the properties of colloid, as well as some more such as viscosity, inherent structural bonds and light refraction. Beside it possesses all biological properties such as growth, development sensitiveness, reproduction, excretion, respiration, etc.

CELL MEMBRANE

The surface of the cytoplasm is bounded by a living plasma membrane, concei
This membrane .
even in protoplasmic masses, such as bacteria and Cyanophyceae whose cellular nature is still open to question. This delicate and living membrane, lacks a strong mechanical resistance, for which reason it is externally reinforced by more coarse and resistant ones. These are the membranes which are generally visible under the light microscope. They should not be confused with the plasma memrbane, as the latter lies beneath them and is not visible under the light microscope. Such layers in animal cells, are composed of protein and carbohydrate compounds with a varying composition. The eggs of some marine animals have an outer covering of gelatinous mucin, a glyco-protein compound. A similar protein conjugate with an aminosugar polymer is found protecting the cells of gastro-intestinal tract. Other carbohydrate modifications are the pectin and cellulose forming the cell wall of plants and chitin forming the exoskeleton of crustanceans. These extraneous coats which surround the animal cell, possess varying rigidity. They are generally considered byproducts of protoplasmic substance, produced for the purpose. Their stability depends on the contents of Ca ions, which stiffens the coat. Further, it has also been suggested that these coats may change the membrane's permeability and protect the cell surface against noxious agents.

THE PLANT CELL WALL

Structure—The plant cells are bounded by non-living cell walls. They are mainly composed of cellulose and are of varying thickness. Parenchyma cells possess relatively thin cell walls whereas the water conducting cells of higher plants, found in xylem tissue possess thick cell walls. The supporting tissue cells may have special thickened places in the corners of their cell walls. Further, the cell wall may be smooth or sculptured,

Primary wall—The outer most wall of a cell is called the primary wall. The primary walls of the adjacent cells do not touch each other directly but are separated by an intervening layer, the middle lamella. This forms the main limiting membrane in the meristematic or growing cells.

2. Secondary wall—A more specialized cell may exhibit additional wall deposits of different materials on the inner surface of the primary wall. This ... lignin or suberin may be ad... mal cells, cutin and cutin waxes may produce an impermeable coating on the surface.

3. Tertiary wall—In the principal water conduting cells (tracheids) of Gymnosperms a tertiary wall becomes the inner most layer of the cell wall. It is often marked with small swellings or warts on its surface. Its chemical composition though not yet known fully, yet it is believed to be composed of a chemical substance known as xylan.

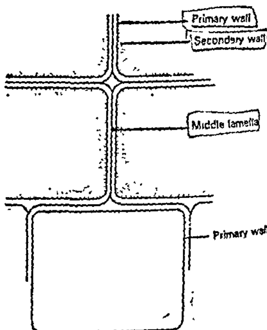


Fig 25. Structure of the plant cell wall.

When the middle lamellae and primary wall develop, there remain certain openings or pores between the adjacent wall. These pores allow the passage for the materials between the cells. In young cells, these pores are small, but they are large in some cells, e. g. tracheids and vassels; both conduct water in xylem tissue. The large pores in the cells are called the pits.

It has been suggested that the elasticity of the cell wall is due to the activity of plant hormones but how they effect the wall, is still not clear. There are evidences to suggest that the middle lamella contains a protein component in addition to carbohydrate

material. This protein may serve to bind the calcium in the middle lamella and the plant hormones may bind the metal ions away from protein. Thus the elasticity of the cell wall increases.

Origin—There are good reasons to believe that endoplasmic

n of middle

;. A sort of

ıe equator of

aughter cells

thereby forming the middle lemella. A new or primary wall arises then on the inner surfaces of the middle lamella in each of the daughter cells.

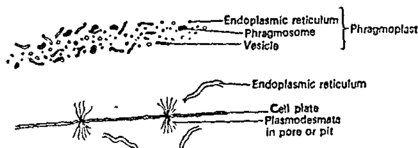


Fig. 26. Diagram showing the formation of middle lamella from the cell plate in a plant cell.

This cell plate is formed by small vesicles whose origin is not fully known, yet they are believed to be produced by Golgi complex. They are about 2·0 mμ in diameter and are rich in pectin. They line up along the equator, to separate the cytoplasm into two parts. Eventually they fuse with one another to form an almost continuous membrane-like structure across the cell. At few points the fusion is not complete, thereby some pores are left out. Plasmodesmata have been observed extending through these pores. Sometimes, elements of endoplasmic reticulum are found associated with plasmodesmata. On either side of these vesicles, several phragmosomes arise. They are about 250 mμ in diameter with an outer membrane layer surrounding rather structureless or slightly granular contents. Their density is much greater than that of small vesicle. When the daughter cells separate, the phragmosomes disappear. It has been suggested that in some way they contribute to the formation of middle lamella. Elements of double membrane endoplasmic reticulum have also been observed congregating at the equator of the cell. They might have migrated from the periphery of the cell,

Chemical nature—The results of chemical studies and physical researches with x-rays on the cell wall have shown that it has a crystalline structure, *i. e.* it is composed of mainly of units arranged in a three dimensional pattern. The primary unit is the anhydrous glucose residue, $C_6H_{10}O_5$. Such residues are united by primary valencies into long *cellulose chains, and these* are linked laterally by secondary forces to form a regular space lattice. The intermolecular cohesive forces result in the formation of larger groupings or micelles.

The primary cell wall is made up of cellulose, a complex carbohydrate substance. The major components of the cell wall is water, about 90%. Such a high quantity of water provides elasticity to the cell wall. The cellulose is the polymer of disaccharide cellobiose, of which the glucose is the basic unit. Thus the long chain cellulose is composed of glucose unit, which are bound as cellobiose molecules, linked at their ends with hydroxyl groups. The number of glucoses residue per cellulose chain may vary upto 3000. The chain molecule of cellulose does not occur as isolated but always occurs in parallel bundles called microfibrils. Each such bundle comprises 2000 cellulose chain molecules giving a diameter of the order of 100-250 A. Sometimes the microfibril join to form macrofibrils.

The microfibrils are losely organised and scattered in the primary wall of higher plants ; in the spaces between them, deposition of other carbohydrate material usually occur. The other material includes hemicelluloses, *pectins*, and *lignin*. Hemicelluloses consist of pentoses (arabinose and xylose) and hexoses (mannose and galactose). The hemicellulose xylan is found particularly in woody *tissue* and as much as 50%of cell wall may be of *hemicellulose in collenchyma*. The pectins are important constituent of the middle lamella. Chemically, the pectin consists of carbohydrate galacturonic acid which is capable of forming the salts with calcium and *magnesium*. The calcium-containing pectin is the main component of middle lemella of most plant cell walls.

The secondary wall is also mainly composed of cellulose to which sometimes deposits of lignin may be found, particularly in more specialized cells and in places where several cells lie side by side. Other secondary wall materials include the cutin and suberin.

The formation of the secondary wall takes place by the apposition of microfibrils and lignin. The fibrils of the secondary wall

are more closely packed than those of the primary wall and are usually arranged in parallel pattern only.

THE PLASMA MEMBRANE

The term plasma membrane may be defined as the cell mem- [illegible] in most animal cells it is the outer limiting membrane. It is supposed that every living cell has a plasma membrane but it is not possible to investigate its structure in every type of cell.

The studies on the cell membrane by X-ray diffraction, polarized light and the electron microscope throw some light on its organization. As visualized by **Danielli** and **Darson** (1952), the [illegible] les set bet- [illegible] rbohydrate. [illegible] bon chains towards each other and with their respective polar gaps arranged outwardly and inwardly. Further the lipid molecules are arranged in such a remarkable fashion that the fatty acid (water insoluble) tail face one another along the double row. The water soluble heads face outward and appear to be at least partially embedded in the outer layers. Recently, **Edward Korn** (1966) has cast doubt on the specific orientation of the phospholipids in the membrane.

Roberton (1962) however disagree with the conclusion drawn by **Danielli** and others (1943 and 1952) that both outer layers of the membrane are proteinous. Although he believes that the layer of the membrane next to the protoplasm is mostly proteinous.

Structure—The plasma membrane consists of usually two layers, *i. e.* the outer and inner layers. The outer layer is dense and made up of protein which is usually absorbed or deposited on the surface. The inner layer consists of bimolecular organisation of phospholipid. The unit membrane as described by **Robertson** (1959) comprise two dense layers, each being 25A° thick enclosing a less dense area of about 25A° across. He also suggested that the measurement of the membrane varies considerably in different mammalian cells. For example, the erythrocyte of the rabbit measures about 215A° thickness. The plasma membrane of intestinal epithelial cell is 105A° in thick, comprising two outer layers each 40A° thick and the less dense layer between 25A° thick (A° or Angstron unit= 10^{-8}cm). In the membrane minute pores of about 50A° diameter or less have been observed. These pores are considerably smaller than

the pores present in the nuclear membrane. The plasma membrane of adjacent cells separate from each other by a distance of 110A° to 150A° with some double cementing substance in between, but the evidences for this are weak.

The following specialization of the plasma membrane are noteworthy. They are either the specialization of the cell surface, or specialization at the base or specialization of contact surface of adjacent cells.

Mi[illegible]
brane in [illegible]
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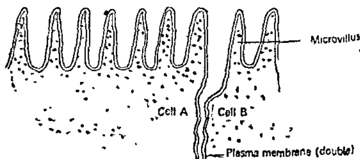


Fig. 27. Plasma membrane showing the microvilli at its surface.

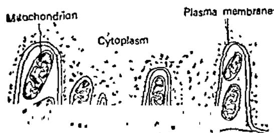


Fig 28. Involutions of the plasma membrane with mitochondria located in the membrane originated chambers.

epithelium can be cited as a very good example of infoldings. The microvilli are generally 0 6 to 0 8μ long and only 100mμ in diameter. They are formed of dense cytoplasmic process covered by the plasma membrane. These are regarded as a device for increasing the effective surface for absorption. A single cell may contain as many as 3000 of them and in a square millimeter of intestine there may be about 200,000,000. The narrow spaces between the microvilli form a kind of sieve through which submicroscopic fat globules pass during absorption. Numerous other cells have microvilli, of course fewer in number. They have been found in mesothelial cells, in the epithelium of gall bladder, uterus,

in hepatic cells and so forth. Between the microvilli, there extend invaginations of the cell membrane from the base to the apical cytoplasm. These structures are supposed to be the pathway by which the fluid can enter in considerable quantity by a process, similar to pinocytosis.

At the cell bases of certain cells, the invaginated membrane forms pockets in which mitochondria are lodged. This close association of the two suggest that the transport of material across the plasma membrane may be made easier by the energy available from mitochondria.

Desmosomes—The membranes of the adjacent cells remain separate and seldom adjoin along a straight line. The intercellular spaces may be wide or narrow. Sometimes the cells are very closely and tightly adjoined to give the impression of direct connection between the membranes. In reality Fawcett (1961) has pointed the direct cent cells during spermatogenesis o rabbit, monkey, and man. However, in many animal cells thickenings appear on the inner surface of adjacent plasma membanes at the point of what was interpreted as an intercellular bridge. From such thickenings, fine filaments, the tonofibrillae radiate which pass through the bridge and enter the interior of the cell. These thickened regions of membranes along with tonofibrillae are referred to as desmosomes. The desmosomes are always located on the inner membrane in each cell, formed by the deposition of some extra material on the membrane rather than the thickening of the membrane itself.The outer membrane of the double layer always ramain uneffected.

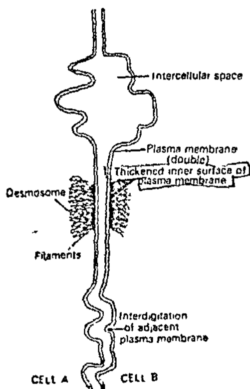


Fig. 29. plasma membrane showing a desmosome and a region of interdigitation of the plasma membrane.

Chemical Nature—Due to, too small amount and size of the single cell membrane, it is however, difficult to do its microchemical analysis. Such a type of analysis is only possible with the mass of cell membranes. The ghost of erythrocytes and also of the bacteria have been subjected to biochemical analysis to determine some of their constituents (Parpart and Ballantine, 1952 ; Dubos, 1947 ; and Miles and Pirie, 1950).

The mass of ghost (erythrocytes releaved of hemoglobin) appears to consist of nothing but cell membrane. The analysis of ghost indicate that they consists of lipid and protein in the ratio of 1·0 to 1·7 by weight. The protein called stromatin, has a high molecular weight and is fibrous in nature.They are also glycoproteins and mucoproteins. It also has relatively large amount of arginine and lysine. In addition to this histidine, tyrosine, tryptophan, methionine and many others aminoacids are also identified. The protein is

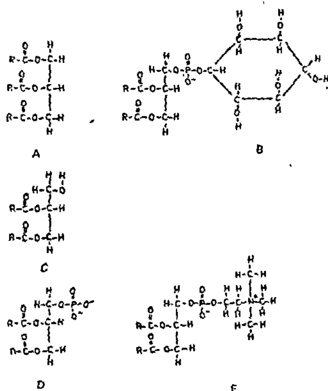


Fig 30. Structure of lipids. A=Triglyceride ; B=Phosphatidyl inositol ; C=Diglyceride ; D=Phosphatidic acid ; E=Lecithin.

acidic in nature and form greater percentage of membrane as compared to lipid components. The lipid are mainly phospholipids and cholesterol. Cholesterol amount in the cell membrane ranges between 15 to 32 percent of the total lipid content. The phospholipids mainly consist of lecithin and cephalin, the amount of which ranges between 55 to 75 percent to the total lipid content. It is further estimated that on an average the cell membrane has 75 to 90 molecules of lipid for every molecule of protein. Parpart and Ballantine, (1952) pointed out that enough lipids are there to cover the surface of R.B.C., at least with 2 or 3 layers. Thus over all five phospholipids have been identified in cell membrane. One is simply phosphatidic acid, *i. e.* diglyceride with the phosphate molecule attached to the glycerol. The other four are more complex being comprising either choline, inositol, eithanolamine or sercine attached to the phosphate groups in addition. The one containing choline is called **lecithin**, is made up of more than half of the total phospholipid content to the cell membrane. Further, the phospholipid molecules, which are made up of glycerol and phosphate, are water soluble. The fatty acid molecules which are tail of the whole structure, are water insoluble. Bell (1962) pointed out the presence of a polysaccharide in the outer layer of the plasma membrane of *Amoeba* which serves to confer some stability to the lipoprotein compound. Parpart and Ballantine (1952) suggested that the structure of the plasma membrane is different in different cells, according to their metabolic requirement and their activity.

Some investigators, disagree with these analysis of the chemical contents of the cell membrane, specially since the nature of the ghost varies with its mode of preparation (Ponder, 1952). For instance it is possible that the erythrocyte ghost includes some of the internal structures upon which the hemoglobin have been found. But, however, the observations from ghost of bacteria generally corroborate those on erythrocyte.

Further Lansing and Rosenthal (1952) using staining methods, however identified the presence of nucleic acid in the membrane of the *Arbacia* egg. Some others have also observed its presence in other cases.

Salts are also present in cell membrane. The micro-incineration test suggest that some of them are present in higher concentration in the membrane, than else where in the cell. Water is also present in it.

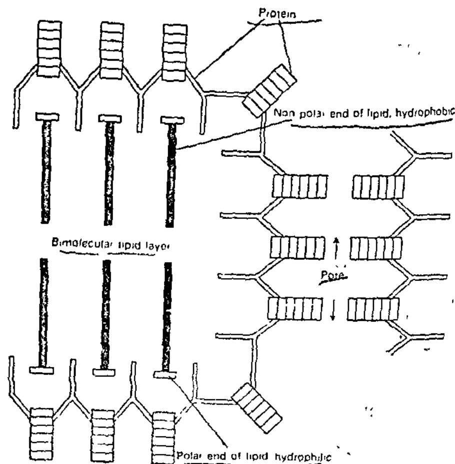


Fig. 31. Molecular organisation of the plasma membrane.

In addition to this, there are evidences suggesting the chemical nature of the cell membrane. First of all **Overton** (1895) suggested that lipids were important in the cell membrane structure. Later **Langmuir** (1917) and **Gorter** and **Grendell** (1925) gave the idea that how lipid molecules are displayed in the membrane. The lipoprotein structure coincides well with the function of permeability and the surface tension of the various kinds. The protein constituents provide the flexibility to the membrane. The protein molecules can fold and unfold with the result the membrane can expand and contract thereby producing molecular spacing, though which molecules can enter the cell from the outside or pass to the outside from inside.

Lowell and Hokin (1965) demonstrated that the phospholipids are not simply inactive structural units of the cell membrane, but

they are secretory in function and they increase the formation of the membrane phospholipids. It has been noted that during secretion there is a striking increase in phosphatidic, inositol, phosphatidyl, ethanolamine and phosphatidic acid ; but not of lecithin. The change in phospholipids is known as **phospholipid effect. Hokin,** *et al*, further demonstrated that the phospholipid effect actually take place within the cell membrane. They work out the **phosphatidic acid cycle.** According to this cycle new membrane is added to the plasma membrane ; by coalescence the membrane breaks down into subunits which are discharged into the cytoplasm. This release of subunits is brought about the hydrolysis of phosphatidyl inositol. As a whole it can be interpreted that the phosphatidyl inositol is the cement holding sub units together ; if it breaks down, it will allow subunits to release from the plasma membrane and its resynthesis allow reasonably the subunits in the intracellular membrane.

Further **J. David Robertson** (1962) has presented evidence that the cell membrane is always a doubled layer unit and he refered it as a **unit membrane.** The unit membrane has been found in all plant and animal cells. After studying structure of endoplasmic reticulum, mitochondria, Golgi apparatus and nuclear membrane, he further suggested that in cell development there is first a mass of cytoplasm surrounded by a surface membrane. The membrane then invaginates to form the various canals and cavities. In addition to this further invegination results in the formation of the nucleus. In this way the cell membrane not only enclose the cell and play the role for the movement of the substances into and out of the cell, but it is also responsible for the differentiation of a very simple structure into the complex unit.

Physical nature

Surface charge—The fact can well be demonstrated that the living cell surface has an electrical change. The movement of the cell in respond to the electric current is thought to be due to the change on the surface of the cell and not to any influence of the internal of cytoplasm. It is most probablythe membrane protein which however, carries the surface change.

Surface tension—In general the term tension involves, the concept of resistance to being stretched. There is however a intermolecular attraction which is generally known as cohesion. Due to the cohesive force,the different molecules oppose to become separate from one another. This opposition or the resistance is known as

surface tension. Thus inside the cell, similar forces, however, operate to hold it together, to maintain the spherical shape, to oppose distortion. Thus, if no cell membrane existed, the cohessive molecular forces would have exerted a degree of surface tension. So when a cell membrane is present, the surface tension is greatly increased.

Functions—The plasma membrane performs several functions. The main function is to regulate the flow of materials into and out of the cell. For the inflow of the material, the plasma membrane should have permeability.

The movement of the water and other substances through the cell membrane, depends on many factors or forces. The different forces include hydrostatic pressure, osmotic pressure and molecular activity. The molecules of water at all temperature except below absolute are in constant movement. Although there are certain compelling evidances that water is never actively transported but rather is dependent upon the relative osmotic state on the two sides of the membrane. In the same way, if a membrane separate two compartments of differing hydrostatic pressure, there will be a movement of water from the high hydrostatic pressure compartment towards the low-pressure compartment. Further the movement of water into and out of the cell is dependent upon the permeability of the membrane and the transmembrane osmotic pressure, *i. e.* the difference between the osmotic pressure of the cytoplasm and surrounding fluid.

Moreover the forces which will influence the movement of solute through membranes are the concentration gradient, electrostatic consideration, partition cofficient and active transport. The cell membrane permeability is altered by change in the environment of the cell. These includes the ionic concentration, presence of narcotics, temperature, radiation, electric current, pH, etc. They all have very great effect on the permeability.

However, the inflow of material into cytoplasm occurs mainly by four processes, *i. e.* Osmosis, phagocytosis, pinocytosis, and active transport.

1. Osmosis—The plasma membrane is often said to be a semipermeable or differentially permeable membrane because water moves through it more easily than the larger molecules. Osmosis accounts for the movement of water through the membrane with the implication that solute move at slower rate, if at all. It means

that the solute would remain isolated on one side of the membrane—this does not occur in living cell. No doubt the smaller molecules like water move with greater facility through the membrane than the large molecules, yet many larger molecules are able to penetrate the membrane at certain times during the life of the cell. As such there should be some other force also,basides osmosis, which can account for the penetration as well as for changes in the premeability of the membrane. Perhaps it is better to say that the membrane is selective permeable and relatively labile in permeability.

2. Phagocytosis—Most cells, receive their food in a state of solution. In metazoans, substances are digested by enzymes in the interior of the digestive tube and after absorption, pass to the internal fluids where they possess molecular dimensions. In some cases, how [illegible] through plasma mer [illegible] (Gr : *Phagein*= to e [illegible] among certain cells of the Metazoa. Among protozoans, phagocytosis is intimately associated with amoeboid motion. Amoeba can ingests large particle and surround them with pseudopodia to form a food vacuole.Among metazoans it is generally a means of defense than serving the cell nutrition. This permits the ingestion of bodies which are foreign to the organism, like bacteria, dust particles and various colloids. Among mammals this property is found very highly developed in the granular leucocytes. The particles become absorbed at the surface of membrane and later on they are taken into the cytoplasm by infolding of plasma membrane.

The process of phagocytosis involve two distinct phenomena : (1) the adhesion or absorption of the particle to the mass of the protoplasm, and (2) the actual penetration of the particle into the cell.

3. Pinocytosis—In addition to the ingestion of solid particles, the uptake of fluid vesicles by a living cell has been observed. This [illegible]

in some way and the membrane that forms the vesicle may become either the part of the endoplasmic reticulum or becomes

reincorporated into the framework of plasma membrane. Proteins can enter this way, was first demonstrated be Mast and Doyle in 1934 in amoebae.

4. Active transport—The movement of ions and molecules across the membrane by mechanism other than osmosis, phagocytosis and pinocytosis is correlated with active transport. It has been defined by Stein (1969) as, 'the movement of molecules or ions in a direction opposite to that of a prevailing electrochemical gradient'. There can be several possible mechanisms of active transport. In one mechanism the transportant (molecule to be transported to the cell) and some chemical component of the plasma membrane form a complex. This complex is then transferred across the membrane and the tansportant is released on the opposite side into the cytoplasm. Another possible mechanism is the metabolic activity inside the cell, as a result of which the number of free ions or molecules decrease as in the transfer of an inorganic phosphate to an organic one (ATP, creatine, phosphate, etc). Thus the number of phosphate ions will become smaller, and in order to restore the equilibrium, an increased passage of phosphate ions from the ouside will take place. The active transfer of the fluid through pores of the membrane can also take place. For all this, energy is needed which is derived from the high energy phosphate bond.

Still another possible mechanism suggests that there exist some substance in the membrane which transport the transportant to the interior of the cell. This substance has been named as carrier which may be an enzyme. A group of enzymes, collectively called permeases is anchored to the outer membrane of the bacterial cell. This carrier, *i. e.* permeases form a complex with the penetrative substance and carry it across the membrane and then releases the substance either inside the cell or at the internal end of the membrane. After the release, the carrier gets the energy from the cell so that it can return to the outer surface to resume its transportation activity. While going back, it can carry ions or molecules from the inner surface and transport them to the outer surface for release.

SUMMARY

Plasma membrane limits the cell from outside in all the case. In plants, in addition to the plasma membrane, there are often other layers over it which encompass the cell *i. e.* cell wall. The cell wall is mostly

made up of cellulose. It or the other extraneous membranes that may surround some animal cells, are quite variable in rigidity. They play very little or no role in determining the movement of substances into and out of the cell, but its function is a protective one.

The plasma membrane is a "sandwich" like structure about 75 A° thick and composed of two outer layers of proteins and a double inner layer of fat. In the case of erythrocytes and intestinal epithelial cells, the cell membrane measure about 105 A° in thickness. Pores of about 8 A° diameter thought to exist. It has been further noted that phospholipdis play an important role in cell, whose activities are secreting. This is known as phospholipid effect. The cell membrane has been referred to as unit membrane by Robertson (1962). He further pointed out that the cell membrane, with all other important works, also play the role in the development of the various internal cellular structures. The surface of most cells has negative charge. The surface tension is quite low about 1 dyne/cm or low.

Several forces act on the movement of water when it move through plasma membrane. The important forces are (1) molecular activity, (2) hydrostatic pressure and osmatic pressure. The factor that determines the movement of solute through the cell membrane are (1) molecular size(2) partition coefficient(3) concentration gradient (4) charges and (5) active transport. In the same way several other activities are shown by the cell membrane as phagocytosis, active transport, pinocytosis, etc. The environmental factors such as temperature, pH, ionic concentration, narcotics, and electric current, etc. affect the permeability of the cell membrane to a great extent.

6

ENDOPLASMIC RETICULUM AND RIBOSOME

ENDOPLASMIC RETICULUM

Endoplasmic reticulum (ER) is a recent discovery among the cellular components and is found in such a variety of forms that it has presented a problem in the terminology to some extent. It was however, discoverd by Garnier in 1897 with the help of light microscope. It is a network of double membrane, distributed extensively throughout the cytoplasm. **Porter (1961)** stated. 'the endoplasmic reticulum is a complex, finely divided vacuolar system extending from the nucleus throughout the cytoplasm to the margins of cell.' In short the reticulum consists of membranes enclosing a series of continuous and discontinuous vacuoles,found in hyaloplasm —the aqueous portion of the cytoplasm excluding all the particulate structures.

MORPHOLOGY

Reticulum is a definite cell organelle rather than artifact. It has clearly been shown by the studies through electron and phase-contrast microscopes. Its form and appearance varies considerably not only in different cells but also in the same cell. It is found in all kinds of cells except the mature mammalian erythrocytes where even the nuclei are wanting. Reticulum normally occur in three main forms : cisternae (lamellae) ; vesicles, and tubules.

Cisternae (lamellae)—They are long and flattened units, 40 to 53 m μ thick and often arranged in parallel stacks. Their arrangement is characteristic in synthetically active cells such as pancreatic cells which produce digestive enzymes. Normally they are found in secretory cells.

Vesicle—They are usually rounded in shape and vary from 25 to 500 m μ in diameter.

Tubules—They are usually more diverse in their shapes than those of cisternae and vesicles. Their diameter range from 50 to 1000 m μ. Normally they occur in non secretory cells.

The three forms of reticulum may appear in a single cell at

the same time or may appear at different times during the cell cycle. There are, however, species differences in the pattern of endoplasmic reticulum as well as differences among tissues or cell types. For example mammalian liver cells show parallel lamellar elements (cisternae), of more or less uniform size during metabolic activity whereas the pancreatic cells show a slightly different arrangement of cisternae, with size difference in the individual units. In *Ambystoma* larva, the notochordal cell shows another pattern in cisternae arrangement of endoplasmic reticulum. It has been noted that there exists some similarity of pattern in cells having the same general functions. In striated muscles, endoplasmic reticulum is always arranged in the form of network of tubules, commonly called as the sarcoplasmic reticulum.

ER may further be classified into two types, i. e. granular or rough and agranular or smooth-surfaced reticulum. *This roughness* is because of the presence of tiny particulate components, called the ribosomes.

In some cells the membranes of reticulum form a continuous array of connecting elements which provide channels for the movement of materials. These reticulum channels may be continous

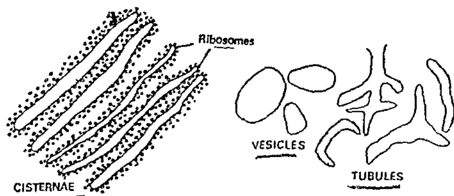


Fig. 32. Forms of endoplasmic reticulum.

with other membranes including that of Golgi complex. Besides, the reticulum membrane is in continuity with the outer of the two membranes that invest nucleus (nuclear membrane). Further, the two are similar from a chemical and structural stand points and that together they comprise an eloberate system for communicating intra and inter-cellularly. Thus it can be concluded that the nuclear

membrane is a special part of the endoplasmie reticulum which for some functional reasons is associated intimately with interphase chromosomes. Or the ER is an extension of the outer membrane [illegible] plasm. However, the pores found in nuclear [illegible] ulum. Sometimes, the invegination [illegible] ic vesicles) instead of being delimited [illegible] go deep in the cell and [illegible] ne continuous with the reticulum. Thus the reticulum and plasma membrane become connected. The reticulum is composed of lipoprotein like that of nuclear membrane and plasma membrane and has the capacity of permeability.

Origin of ER—As regards the origin of ER, many controversial theories have been put forwarded It has been suggested by Palade that ER originates as an infolding or infoldings of the cell membrane. Others suggested Nebenkern as a possible region in the cytoplasm where ER is formed. The Nebenkern is made up of dense aggregations of concentric rings. Such aggregations have been noted in close association of nuclear membrane and it has been suggested that these membranous rings are the pealing off the nuclear membrane. It has been proposed that pealing off the membranes from such a germ center (Nebenkern) has given rise to the ER. There are number of authors who think the ER, associated with mitochondria and the former is being formed on the surface of the mitochondrial membrane, which finally split off. However, there is nothing more to suggest that the mitochondrial membrane might not physically form the ER, but they decidedly supply energy, necessary for the production of ER—membranes. It can be concluded that the origin of ER is still a problem which is far from solved.

RIBOSOME

Palade in 1955, discovered the small dense particulate components in the cytoplasm to which the name ribosomes or ribonucleoprotein granules (NRP) has been given. The ribosomes are ubiquitons (existing every where) elements found in all cells that synthesize the protein. This is composed of electron-dense granules of macromolecular dimensions (100 to 150A°) found frequently associated with the membranes of endoplasmic reticulum. In many cases this association is very tight. Ribosomes are found attached to the outer surface of the membrane and in a tangential view, may show certain definite patterns like rosettes, spirals and circles. This association has given rise to a number of different nomenclatures, such as

"rough-surface endoplasmic reticulum' or 'granular reticulum' as opposed to the 'smooth-surface' or 'agranular' reticulum. In the later the membranes are devoid of ribosomes. Though these two components are closely associated in many cells, yet they are very different in their properties, chemical composition and probably in their functions. Normally they are found attached to the cisternal type of endoplasmic reticulum and are found in cells active in protein synthesis. They not only appear along the membrane surface but in many cells, like meristematic cells of plant tissue they are also found scattered more or less free in hyaloplasm. The bacteria which seem to possess little of no ER are nevertheless rich in ribosomal particles.

They contain roughly 40% to 60% RNA and 60% to 40% protein. The RNA is of very large molecular weight. The structure and appearance of ribosomes are roughly dependent on the presence and on the amount of magnesium. In the absence of magnesium in the medium, the large particles fall apart to present a family of smaller particles. Bacterial ribosomes perhaps exist in a cell as 100S or 70S particles (S=Svedberg unit) but they can be broken to smaller ones. Ribosomes from mammalian cells exists probably as 80 S particles which can be split to 40 S particles. How the smaller particles combine to form large ones is not fully known, but Mg^{2+} complexing is thought to be involved. It is also not fully known that which of these two are active in protein synthesis. The proteins of ribosomes appears to be basic in nature.

Ergastoplasm—There are certain regions in the cytoplasm that stain with basic dyes, such as the nucleus. To these regions *various names have been given like chromidial substance, basoplasm,* ergastoplasm and so forth. The term ergastoplasm was given by Garnier in 1887 (Gr : *ergazomai*=to elaborate and transform). The ergastoplasm includes basophilic regions of the ground cytoplasm such as the Nissl bodies of the nerve cells, the basal cytoplasm of serous secreting cells (pancreas, chief cells of stomach), the basophilic clumps of liver cells, etc.Studies of Caspersson (1955), Brachet (1957) and others have demonstrated that the basophilic nature of ergastoplasm is due to the ribonucleic acid. After the discovery of ribosomes (Palade, 1955) it became appearent that the stainning property of ergastoplasm is dependent on these particles. These findings have clarified the relationship that the endoplasmic reticulum and the ribosomes has with the fine structure of the ergastoplasm,

Thus ergastoplasm also refers to the well developed endoplasmic reticulum and the associated ribosomes in some parts of metabolically active cells.

In the acinar cells of the pancreas, ergastoplasm is very abundant and is distributed throughout the cytoplasm. It appears as flattened sacs (cisternae) with a high degree of orientation and a rather regular parallel array. In the liver cells stacks of sacs with the associated RNP particles near the nucleus or in the midst of the cytoplasm are found at the place of the basophilic bodies. In nerve cells, the Nissl bodies show a high degree of orientation of the flattened sacs with numerous RNP particles.

Myeloid bodies—There are also some specialised membrane elements located in certain kinds of cells; out of these myeloid bodies are worth mentioning here. These are the specialisation of endoplasmic reticulum and are found in pigmented epithelial cells of the retina. It consists of a compact arrangement of vesicles and tubules, very tightly packed, situated near the basement membrane of the cells. They are devoid of ribosomes as such classified as smooth or agranular. They are light sensitive regions in the cell and play some role in photoreception.

Microsome—Microsomes are not the normal structural units of the cell,but represent bits and pieces of the endoplasmic reticulum with associated ribosomal particles removed from the cell. These can be removed from the cell by high speed centrifugation. Claude (1941) was the first who succeeded in separating such a fraction from the liver homogenates and termed them first "small granules" and later "microsomes". This fraction is rich in RNA and shows a high rate of protein synthesis than in non ribosomal endoplasmic reticulum. In reality there exists a sort of close correlation between the RNA contents of the microsomal fraction and the rate of protein synthesis. If the microsomal fraction is treated with ribonuclease (an enzyme that inactivates the RNA), the incorporation of labelled amino acid into protein is inhibited (Zamecnick and Keller, 1954). The microsome is a heterogeneous fraction consisting without any doubt not only the ergastoplasmic components, *i. e.* vacuoles with RNA particles but may also contain Golgi membrane, ruptured plasma membrane, and other cell fragments.

FUNCTIONS OF ENDOPLASMIC RETICULUM

1. Protein synthesis—There are good evidences suggesting the involvent of ER or its derivative, microsome fraction in the protein

synthesis (Palade, 1958 ; Sickevitz, 1959). Microsome fraction possesses the same three main components which are recognizable in rough ER and particularly in the ergastoplasm. These components are the membrane which corresponds to the ER membrane, particles or ribosomes attached to the outer surface, and the vacuoles which are either amorphous or granular. Further, it has been demonstrated experimentally that the high RNA contents of microsomes is localized exclusively in ribosomes and the membranes probably contain the lipid and chemical components characteristic of this cell fraction. These ribosomes are related to protein synthesis. It has been pointed out that the free ribosomes are not as active in protein synthesis as are those associated with ER. This suggests that the membrane contributes in some may to the efficiency of the process.

2. Cellular metabolism—The membrane of the reticulum provides an increased surface for metabolic activities. This may [illegible] iter [illegible] me with its substrate. The smooth form of reticulum is generally found in cells that are active in the synthesis of steriod compounds. (cholesterol, glycerids) and harmones (testosterone and progesterone). It is also found in pigmented epithelial cells of the retina, which are involved in the metabolism of vitamin A. Glycogen storing cells of the liver contain the smooth tubular elements of the endoplasmic reticulum. All these instances point out to the active participation of the membranes in cellular metabolism, apart from the protein synthesis associated to ribosomes.

3. Infracelluar transport—The reticulum acts as segregation apparatus and collects matters that are synthesized for delivery to other parts of the cell or to the exterior. It may be said to act also as a kind of [illegible] involved in the import, export and [illegible] is an active intake of fluid from the surroundding medium. In numerous cells it is possible to see invegina tions of the plasma membrane forming pockets that are associated with intra-cytoplasmic vacuoles. These observations suggest that the plasma membrane may flow actively and become incorporated to form part of the reticulum in the cytoplasm.

plasm. This is quite evident from the figure 34 (A to F). A similar mechanism, but in the reverse direction, *i. e.* F to A can effect the transport of a particle (secretion) from the interior of the cytoplasm to the outside. Sometimes,a continuation exists between the reticulum and the nuclear envelop which suggest that membrane flow may also be active at this point. This hypothetical flow may provide one of several mechanisms for transmission of nucleoproteins from the nucleus to the cytoplasm.

4. ATP synthesis—ER membrane is a site of ATP synthesis in the cell. The ATP is used as a source of energy for the intracellular transport of meterial or RNA metabolism involving ribosomes.

5. Intracellular impulse conduction—Specialised arrangements

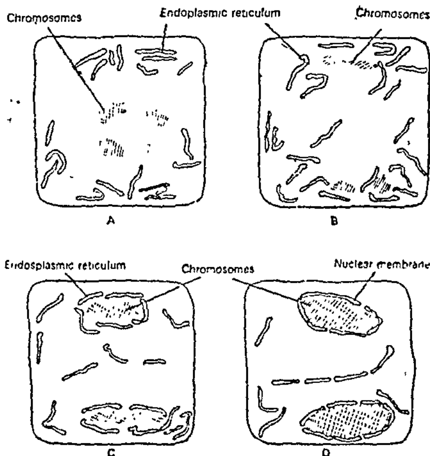


Fig. 35. Diagrams of the successive stages of the cell division, to show the formation of nuclear membrane from the ER (Diagrammatic)

of the E R, such as the sarcoplasmic reticulum may allow for the transmission of impulses or excitations intra-cellularly from the sarcolemma into the deep regions of the muscles (Portor, 1956).

6. **Behaviour of ER during cell division**—(a) During cell division, some of the elements of r [illegible] in the formation [illegible] These vesicles [illegible] ase starts, where they are indistinguishable from the elements of ER. From the polar ends of the cell, elements of ER as well as the fragmented vesicles migrate into the regions around the chromosomes, which are grouping at the poles, Most of the these elements of ER join or fuse around each group of daughter chromosomes to form a new nuclear envelop.

(b) The bits of ER help to some extent in the formation of cell plate during the cell division in plants. It is believed that the elements of spindle ER that do not take part in the formation of nuclear envelop, move down into the interzone and reach the equator of the spindle. Here, the advancing margins reticulate to form a lattice of microtubules. Within this lattice, the cell plate first appears.

7. In the formation of secondary cell wall in plants, certain enzymes and metabolites may be carried by the reticulum to the region of wall synthesis.

8. There are evidences to suggest that the ER contributes in several ways to the development of the amphibian embryo.

SUMMARY

Endoplasmic reticulum (ER) is another cytoplasmic component which has been defined by Porter as "a complex, finely divided vacuolar system extending from the nucleus throughout the cytoplasm to the margins of cell." It is a network of channels. These channels may be smooth-surfaced or rough-surfaced. In the later case they are covered with tiny, spherical structures called ribosomes which contain a large amount of RNA. The cells that produce a large amount of protein always contain large amount of RNA. About 90% of a cell's

RNA is found in ribosomes. It is thought, therefore, that the ribosomes manufacture proteins. ER normally occurs in three forms, *i.e.* cisternae, vesicles, and tubules. How it originates in the cell, is still a problem to be solved. There are divergent views. Myeloid bodies are specialized ER and are found in pigmented epithelial cells of the retina. Microsomes represent the bits of ER with associated ribosomes. ER subserves several functions : (1) It helps in protein synthesis as the free ribosomes are not so active in protein synthesis as are those associated with ER. (2) It provides an increased surface for metabolic activities. (3) It acts as segregation apparatus and collects matters that are synthesized for delivery to other parts of the cell or outside. (4) During cell division it contributes in the formation of new nuclear membrane after karyogamy.

GOLGI SUBSTANCE OR GOLGI COMPLEX

The Golgi complex has been the most controversial of cellular constituents, right from the time of its discovery to the present day. Several interpretations have been attributed to this cytoplasmic meterial. Part of the difficulty lies in the lack of refinement of techniques for the observation of such cytoplasmic structures. Further, it is a matter of great variation, consequently its presence could not be universally accepted by the scientists in some common forms. But now, its existence has been clearly demonstrated with the electron microscope and by the use of phase microscopy.

History—It was first of all discovered by Golgi, an Italian neurologist in 1898, after whose name it owes the name. He detected it by using the method of silver impregnation in nerve cells of the cat and the barn owl. Impregnation with silver salts or osmium tetroxide detects the presence of lipid material. The use of the vital stain, neutral red or methylene blue produced stained droplets in the cell which were considered by some workers to represent a precursor to the Golgi complex as seen in the cell, fixed with silver or osmium. Due to great variability in appearance and the observations of similar patterns of morphology with numerous chemicals applied on the outer side of the living system, many workers regarded the so-called Golgi complex as an artifact resulting from the methods used to show its presence. Further, Golgi material has been referred to occur as network (reticula) but no such network has been observed in living cell. This provides an additional support to the idea that the Golgi complex is an artifact of fixation.

In most animals, there is a region or portion in the cytoplasm which can reduce silver nitrate or/and osmium tetroxide. Most workers have identified this region but often they have described elements which are not characteristic, depending upon the particular tissue, method, and optical instrument employed in their studies. Baker (1957) could locate this region by using Sudan Black stain and suggested that the variations in the morphology are due to a series of changes in form during the activity of the complex. However, similar

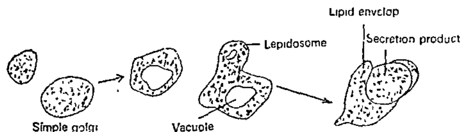


Fig. 36 Morphological changes in Golgi complex during secretion.

changes have been observed with such substances as gum arabic or Toluidine Blue coarcervate in the absence of Golgi.

Nomenclature—Various names have been given to this complex by the various workers, such as **dictyosome, lipochondria, iodiosome, Golgi body ; Golgi substance, Golgi apparatus,** and **Golgi complex,** but generally the name **Golgi** is used for the material in vertebrates and dictyosome in invertebrates. Though the objections have been raised for the use of Golgi for all cases but the term has so wide-spread usage that the two terms, *i. e.* Golgi and dictyosome are used interchangeably to identify the structure in both animals and plants. Further the name"apparatus,"usually given is confusing as it suggests a definite relationship with the physiologic processes of the cell. It appears more appropriate to use the name 'Golgi complex' or "Golgi substance", referring to a material having special properties. The Golgi complex can be considered as a differentiated part of the ground cytoplasm of the cell. It is very difficult to observe the complex in the living cell as its refractive index is similar to that of hyaloplasm. The use of electron microscope has made clear some of the facts about the complex and now its submicroscopic structure is almost well established. However, much less is known about its biochemical properties and probable functions.

STRUCTURE

Morphology (shape, size and position)—The shape of Golgi is quite variable in somatic animal cells ; even in the same cell, there are variations with functional stages. However, the shape is characteristic for each cell-type. In some cases it occurs as a dense reticulum of anastomosing trabeculae while in others as an irregular fenestrated plaque, a ring, hollow spheres united together. In nerve cells it occurs as a reticulum of wide meshes around the nucleus.

Similarly its size is also variable. It is small in muscle cells but quite large in the nerve and gland cells. They appear to be linked to the functional state for example hypertrophy in hyperfunction and atrophy in hypofunction. Generally it is well developed in an active cell but when the cell grows old, the complex gradually diminishes in size and finally disappears. The position of Golgi is relativaly fixed for each cell type.In the cells which are of ectodermal origin, the complex is polarized, *from the time of the embryonic* state, between the nucleus and the periphery. In the secretory exocrine glands, it is generally found between the nucleus and the excretory pole. In endocrine gland cells, its *polarity* is variable except in thyroid where it is oriented towards the centre of the follicle.

In impregnated preparation, it is found to be composed of two parts, an external argentophilic and osmiophilic part (**externum**) and an internal argentopholic and osmiopholic part (**internum**). The latter is believed to be richer in water and is less dense than the external part.

Submicroscopic organisation—Electron microscope observations of tissue sections demonstrated that the Golgi of animal cell has a

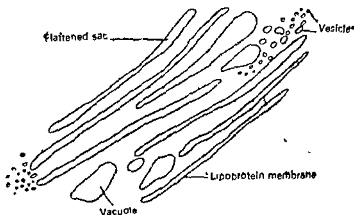


Fig. 37. Structure of the Golgi complex.

definite and characteristic submicroscopic organisation. This permits its identification among the other cytoplasmic components. The complex comprises the following morphologic components.

1. Flattened sacs or cisternae.

2. Large, clear vacuoles.
3. Clustures of dense vesicles.

1. **Flattened sacs**—The flattened sacs or cisternae are similar to the smooth surfaced ER. and appear in the section as dense parallel membranes. In the exocrine cells of mouse, the stacks are arranged in groups ; each group comprising several membrane pairs, separated from the neighbouring group by a space of about 130A°. Each membrane is roughly 60 to 70A° in thickness and the two membranes of the pair are 60 to 90A° apart. The space between the two membranes can however vary from 50 to 200A°. The packed cisternae are often arrayed concentrically, enclosing regions of the cytoplasm filled with numerous large vesicles.

2. **Large vacuoles**—The large vacuoles are clear and generally lie at the edge of the Golgi. They represent the modified and expanded flattened sacs, in which the two membranes of the sac are more widely separated and the vacuolar space has enlarged. In some cells (duodenal epithelium, liver and pancreas) these vacuoles may contain dense masses or granules.

3. **Small vesicles**—The small vesicles, of about 6000A° are intimately associated with cisternae and may show continuity with them. They arise from flattened sacs by budding or pinching off of the sacs. These budded particles remain dispersed in the surrounding hyaloplasm.

Further, the Golgi membrane lacks the dense ribonucleoprotien particles, *i. e.* ribosomes which may found in contact with the membrane of the ER. and ergastoplasm, Palade (1955) has described in some cells, the continuity between the membranes of both the systems (Golgi and ER). According to Palay (1958) it is probable that both the membranes of Golgi and ER. belong to a family of membrane—limited vacuoles that permeate the cytoplasm and are in dynamic equilibrium with one another as well as with the plasma and nuclear membranes.Because of all this and partly because of the staining similarities between the two,it has also been suggested that Golgi system has derived from the elements of the endoplasmic reticulum but this assumption lacks experimental proofs.

Inspite of all this, the present tendency is to consider the Golgi as a distinct morphologic entity within the ground cytoplasm, different from other cytoplasmic components.

Chemical composition—For a long, the Golgi complex was

considered to be composed of lipids. But now it has been confirmed that it always comprises equal amount of phospholipid and protein. The membrane is made up of lipoprotein alone. Baker observed the presence of lecithin and cephalin in the Golgi complex of nerve cells, whereas Cain found carotenoids in the complex of *Limnaea* and *Planorbis*. He further confirmed the presence of fatty acid, lipine in the complex. According to some authors, the protein part, is concentrated, particularly in the internal zone of the Golgi. The most significant biochemical activity of the Golgi complex is due to the enzymes, collectively called **nucleoside diphosphatase**. This enzyme hydrolyzes the diphosphates of uridine, quanosine, and inosine in certain plants and animal cells. This activity is characteristic of Golgi, but also observable rarely in the endoplasmic reticulum and nuclear membrane. Recent studies have shown that the accumulation of secretory products in Golgi vacuoles lead to the formation of lysosomes—another cytoplasmic structure.

FUNCTIONS

A lot of work has been done on Golgi since its discovery, but few facts are known about its functions. Most of the functions attributed to it are based either on indirect observations or on more or less hypothetical deductions.

1. **Golgi complex and secretion**—It has been suggested that ... secretion. It ... developed ... elationship ... ls, salivary glands, and in the exocrine pancreas activated by secretin. Later

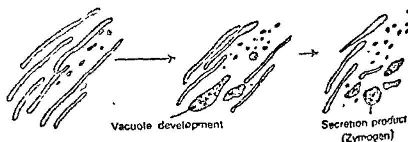


Fig. 38. Formation of the secretion product from the Golgi complex.

on other workers confirmed these observations. De Robertis (1935) presented a typical example. He found in the follicular cells of oocyte that there are present fuchsinophilic secretory granules in intimate association of Golgi. These later become either completely surrounded by osmiophilic ring or by a reticulum with nodal points. Finally the Golgi breaks apart and it has been seen that the secretion granules have osmiophilic parts attached to the surface. Similar conclusions have also been observed by Bowen (1929) in exocrine gland and by Carmer and Ludford (1925) in bilary secretion anp so forth. It has been attributed that the Golgi complex acts in secretion as, "a condensation membrane for the concentration in droplets or granules of products elaborated in other locations that diffuse through the cytoplasm". These products may be lipids, yolk, bile, enzymes, hormones, etc. According to this theory, the Golgi complex would act by surface action and would not intervene in synthesis or in the transformation of products.

Emmel (1945) observed that in the cells of intestinal epithelium, alkaline phosphatase is more concentrated in the Golgi complex. Similarly acid and alkaline phosphatase were observed in the Golgi region of several kinds of epithelial cells by Deane and Dempsey (1945). These facts are suggestive of a participation by the Golgi complex in metabolic processes.

Some workers have emphasized the fact that some secretory granules are selectively collected within the Golgi vesicles (Palay, 1958). These are normally first formed within the small vesicles of Golgi substance found near the nucleus, then the secretory droplets grow and move towards the periphery of the cell for final delivery. During the maturation of these secretory granules, however, the water of the products is removed by the Golgi membrane. By this function, the secretion product becomes packed in a more concentrated granule that is used for storage and final delivery through cell surface.

There are a lot of evidences, indicative that they take little or no part in the synthesis of secretory products. The products of synthesis in other parts of the cell accumulate with in the small vesicles or large vacuoles of Golgi complex. In some cells, the products are carried by vacuolar system of ER to the Golgi sac. In still others, small vesicles may collect and fuse to form larger vesicles or vacuoles. No doubt that there exist continuation between ER and Golgi membranes but it has been attributed that there

exists transitional elements inside the cytoplasm where the transfer of products occurs. This transitional element is the smooth surfaced ER which may give rise to Golgi vesicles on the accumulation of synthetic products. It has been further suggested that whatever be the mechanism for the formation of secretory granules, the later are always surrounded by membranes derived from Golgi complex. When the granule has attained the maximum size, its membrane fuses with cell surface through an opening created at the cell surface.

2. Formation of acrosome during spermatogenesis—During the maturation of sperm, the complex plays a role in the formation of acrosome (Burgos and Fawcett, 1955). In early stages, the Golgi complex appears as a spherical body, comprising flattened sacs arranged in parallel stacks and numerous small vesicles. The latter always pinched off from the flattened sacs. As development proceeds, the complex becomes irregular in shape and large vacuoles are formed by dilations of flattened sacs. In the centre of these large vacuole or vacuoles is/are present a dense granule, the proacrosomal granule. This granule seems to be the secretory products of the complex that grows within the enlarging vacuole. This vacuole+granule approaches the anterior pole of the nuclear membrane, constituting acrosomal granule. With the elongation of the spermatid, the acrosomal vesicle spreads over the nuclear surface and finally collapses with the nuclear membrane, forming

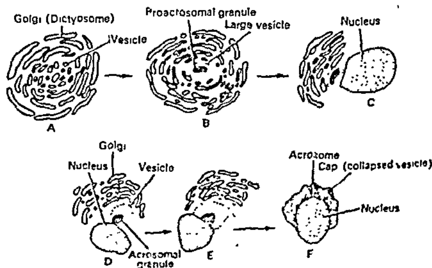


Fig. 39. Diagrams indicating the sequence in the formation of acrosome from the Golgi complex.

the [illegible] wh[illegible] cer[illegible]

3. It activates the mitochondria to produce the ATP which is utilized in respiratory cycle, nervous transmission, and nucleic acid and protein synthesis ; the later occurs in the vicinity of the endoplasmic reticulum.

All the functions described above are closely associated with its secretory activity but what is the functional significance of Golgi in the non-secretory cells and particularly in the nerve cells, is still to be worked out. It has been thought that the Golgi may intervene in the secretion of fats, the elaboration of Nissl bodies, the metabolism of carbohydrates, etc., but it is safer to say at present that up till now there is no satisfactory theory to explain the functions of Golgi complex in a general form and for all the cells.

SUMMARY

Like ER, the Golgi is also a double membranous vacuolar channels which traverse the cytoplasm, may be in continuation with the former. With ER it differs in having no ribosomes associated with the membranes. It was first of all discovered by Golgi, in 1898 in the nerve cells of the Barn Owl as a network of threads when he stained the cell with silver nitrate or osmic acid. For this discovery and also for his investigations on the histology of nervous system, he was awarded Nobel prize in 1907. For long its presence could not be asertained in a cell but now its existence has now been clearly demonstrated with electron microscope and phase microscopy.

Various names have been proposed to this structure but these days only Golgi or Golgi substance or material is wide spread. In a cell its shape and size are variable but its position is relatively fixed for each cell-type. It is small in muscle fibres but it is large and well developed in secretory cells and in nerves. The cannals of Golgi are of different shapes, usually consisting of flattened sacs associated with small vesicles and vacuoles of large size. The surface is covered with double membrane,

each having different staining properties. The inside, appears to be of fluid consistency.

Chemically, the membranes appear to be formed of phospho-lipids, proteins and a number of enzymes. They are very low in nucleic acid contents.

It has been suggested that the *Golgi* act as a sort of intra-cellular pump that regulates the movement of fluids in the cell and the expulsion of secretory products from the cell. It is believed to play a part in the formation of acrosome of the sperm during maturation of the latter. It is also suggested that it activate the mitochondria to produce the ATP.

There exist similarities in the membrane that bound the ER, Golgi, mitochondria and nucleus. Although these membranes differ from one another to some extent but their general similarity with plasma membrane and the similarity of the connections of the channels of ER to the surface of the cell suggest plasma membrane as the possible source of all the membranes. Evidences are still required for this.

8

MITOCHONDRIA

The **mitochondria** (Gr : *mitos*=filament ; *chondros*=granule) hich are generally known as the powerhouse of the cell, are small odies found in the cytoplasm of the cells. These are the site of the hemical events that supply the energy to the cell. As usual, they ere also once considered an artifact of fixation but now it is said hat they "certainly exist" in almost all the animal and plant cells. hey can be easily identified, even with a light microscope because hey are large cytoplasmic organelle.

History—Altmann (1894) was the first to describe the presence f these particles in a cell. To these particles he gave the name f "bioplasts". Prior to him,several other authors like **Flemming** and olliker knew about them but their work remain in dark untill **Benda** 1897) demonstrated anew the structures described by Altmann and lemming. He called them **mitochondria**.

Terminology—According to **Cowdry(1924)** more than 50 names ave been given to this structure. These are called **mitochondria** in nglo-Saxon countries. In latin countries they are called **chondriome**, hen present in group ; each one of them is called **chondriosome**, and he names **mitochondria** and **chondriocont** to the granular and filamentous forms respectively.

Origin—Early workers like **Meves** considered mitochondria to e carrier of hereditary factors which propagate by fission like chromosomes but no such division has been observed.Gey(1956)suggested hat they originate from pinocytosis vacuoles but no direct observation upporting this could be made by other workers. **Robertson (1959)** uggested that mitochondria may arise from cell membrane with hich they may retain the connections. Because of the similarity in norphology between mitochondria and plastids, the former were uggested to originate from the later. But it has been confirmed hat they never arise from plastid. **Hoffman and Grigg (1958)** suggesed its origin from the nuclear membrane. **Ehret and Powers (1955)** uggested the nucleolar origin of mitochondria because nucleoli and nitochondria had similar appearance in thin section of *Paramecium*,

Some authorities believe that they arise from the microbodies found scattered in the hyaloplasm. These microbodies possess the same structure, *i. e.* a single outer membrane, dense matrix and a few crystals. According to them the microbodies accumulate during the regeneration of tissue and by and by through a series of transitional stages, these develop into fully formed mitochondria. Most of the investigators agree with this explanation. They have also been suggested to arise from microsomes but this view is no longer held.

MORPHOLOGY

Shape—The shape is variable but is characteristic for a cell or tissue type, this too is depenbable upon environment or physiological conditions. A typical mitochondrion is a sausage-shaped body but in the living cells they may assume the shapes like that of filaments. They may be granular. They may swell out at one end to become club-shaped or hollows out at one end to assume a shape of tennis-racket.They may become vesicular by the appearance of central clear zone. Rod-shaped mitochondria are also observable.

Size—The size is also variable. In most cells the rod-shaped mitochondria are 0 5μ to 1μ in diameter and of variable length, reaching a maximum length of 7 μ. The different physical and chemical factors such as pH, cell environment and osmotic pressure have some influence on the size and shape of mitochondria. In acid pH they tend to become vesicular.

Distribution—The distribution is in general uniform, but there are many exceptions to this rule. In the cells of kidney they are aggregated and occur in the folds of the basal region near the plasma membrane. In *Paramecium*, they lie just beneath the cell surface. *In* some cases,they are aggregated about the nucleus or in the peripheral cytoplasm. Such margination as well as the perinuclear accumulation may be found in normal cells, but is more frequent in pathologic conditions. They have also been seen aggregated in a radical form about the centrosomes. During cell division, they accumulate about the spindle and upon division of the cell, are distributed more or *less* equally among the daughter cells. They can move freely in the cytoplasm but in some cells they have a permanent location.

Orientation—In some cells, the mitochondria present a more or less definite orientation. Thus in the hepatic cells of fish and amphibia, they are oriented along the axis which unite the *blood* capillaries with the bile capillaries ; in the epithelial cells they are generally oriented in the baso-apical direction, In the leucocytes,

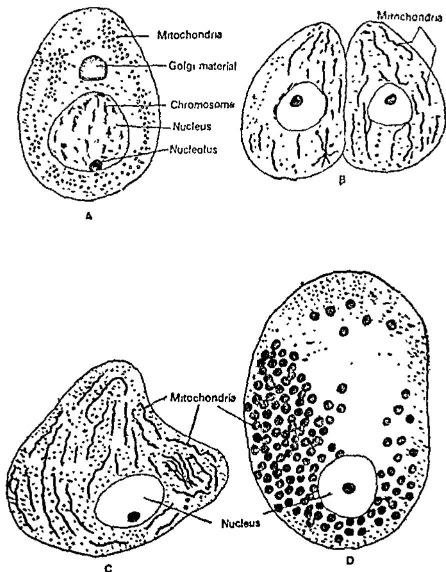


Fig. 40 Different shapes and sizes of mitochondria. A—primary sporocyte in the rat ; B—cell of mammalian kidney ; C and D—liver cells of turtle.

they are radially arranged. Pollister (1941) has suggested that this orientation of mitochondria depends upon the direction of the currents of diffusion within the cell and is intimately linked to the submicroscopic structure of the cytoplasm.

Number—The number of mitochondria in a cell is also variable. According to Junqueira (1951) there exists a direct correlation between their number and secretory activity in salivary gland cell,

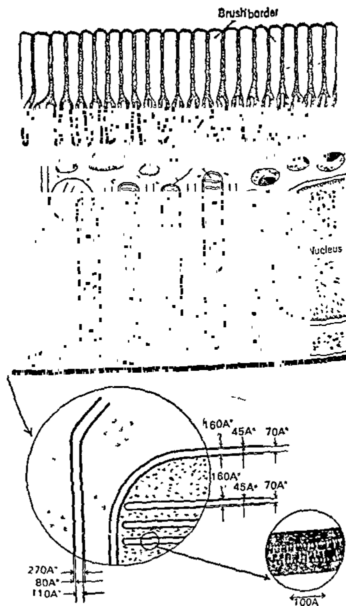


Fig. 41. Structure of the mitochondria in a cell of the kindney of rat. In the mdidle, the circled area of the top figure has been enlarged to show the intracellular cytoplasmic membrane and a portion of the mitochondrion with its internal cristae. At the bottom, the encircled area of the middle figure crista has been enlarged to show its layered protein-lipid layer being the internal one (Diagrammatic)

Amoeba, *Chaos chaos* has about 500,000 mitochondria whereas in the rat liver cell these are only 500. It has also been noted that they vary in number within the same tissue. For example, the average number of mitochondria in a liver cell of rat is 1000 but they may be 2500 per cell. In sea urchin the number varies between 14000 to 150,000 per cell.

STRUCTURE

A typical mitochondrion is a sausage-shaped body, about 15,000 A° long and 5,000 A° in diameter. It has a double layered wrapper with an outer and inner membranes, having watery fluid in between them. Each membrane is a typical unit membrane, being about 60 A° thick. The two membranes are normally 60 A° to 80 A° apart. In between them remain filled the fluid rich in coenzymes.

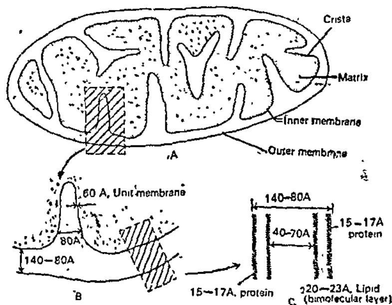


Fig. 42. Structure of the mitochondrion in cross-section (A) ;
B— dimensions of the area marked in A
C—dimensions of the area marked in B.

Extending from the inner membrane into the interior of the cavity, there are present a series of folds called mitochondrial crests or cristae. The cristae may be arranged parallel to the long axis of mitochondrion (striated muscles and neurones) or branched to form a network of connecting chambers (human leucocytes, some

protozoa and parathyriod gland). They may have tubular arrangement (adrenal gland cell) or arranged concentrically inside the matrix (certain spermatids). The term villi have been given for the interlacing cristae in *Amoeba*. When they are in large number as in muscle tissue, the matrix is reduced whereas the matrix is much in those mitochondrion where their number is less.

Thus there are two cavities in a mitochondrion, the one mitochondrial matrix and the other between the two membranes of the wrapper. The matrix is generally homogeneous but in some cases, there are present a number of granules in it, whose density varies in the different cells. In some cases it may show finely filamentous material.

Particles—The electron microscope has revealed the presence of very small particles, adhered to the outside of the outer membrane and the inside of the inner membrane. They were first described by Humberto Fernandez-Moran. The particles of the two membranes differ considerably. The outer membrane particles appear as simple spheres, packed closely together in such a way to give a rough or

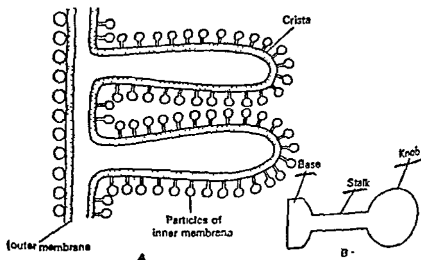


Fig. 43. Mitochondrial wall with attached particles (A); In the Fig. (B), one particle of the inner membrane is shown highly magnified.

pimpled appearance to the surface. The inner membrane particl has the more complex configuration. In some cells, each partic seems to comprise a base, a stalk and an head. The base which i attached to the inner membrane has about 80A° diameter.. Th

stalk is 50A° long and 30A° wide where as the head has the same diameter as does the base. The length of the entire particle measures about 160A°. They are also packed densely. These particles play a major role in the functioning of mitochondria.

Degeneration of mitochondria—Mitochondria are labile structures and can be altered by the actions of various agents. They may fragment into smaller ones. These fragments may swell or may accumulate dense material in them. These altered structures, however, can be reverted to the normal as indicated in the figure 44. If the alterations reach to a certain point it may be irreversible. The

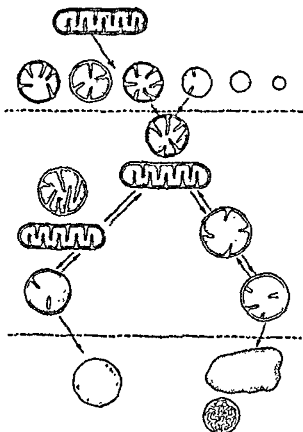


Fig. 44. Diagram showing changes in mitochondria. Process of fragmentation of elongated mitochondria (UPPER), reversible changes of swelling and accumulation of material in mitochondria (MIDDLE), and irreversible changes of swelling and accumulation, *i. e.* degeneration phenomena (LOWER).

degeneration of mitochondria includès these irreversible transformations. The same figure 44 shows some of the degenerative changes that have been observed. These are usually of three types :

1. Fragmentation into granules.
2. Intense swelling with transformation into large vacuoles.
3. Large accumulations of materials with transformation of mitochondria into hyalin granules. This frequently ends with the death of the cell.

Sometimes fusion of mitochondria may occur forming large bodies, the **chondriospheres** (Bourne, 1951). This is also a case of degeneration.

CHEMICAL COMPOSITION

High resolution studies have shown that the mitochondrial membrane has a molecular organisation, similar to that of plasma membrane. Each membrane is made up of two main substances, besides the attached particles. These are proteins and the lipids. The proteins are the main constituent and is roughly 4/5th of the dry weight of the membrane. In its usual form it is insoluble. Analysis of the constituent aminoacid shows that half of them have paraffin-like side chains which are insoluble in water. These side chains are joined together by hydrophobic bonds. The hydrophobic

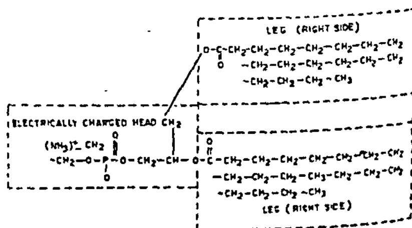


Fig 45. Phospholipid molecule, that make the membranes of mitochondrion. Its shape is some what like a clothespin. The head (LEFT) consists of an electrically charged group of atoms ; and the two legs (RIGHT) are long hydrocarbon chains.

bond is fairly weak but as, a large number of such bonds are involved in joining the chains, it gives the membrane a high degree of stability.

The structural proteins can be broken down into subunits or monomeres by various reagents like acid or alkali. These reagents weaken the hydrophobic bonds with the result the protein breaks up into subunits. These subunits are soluble in water.If the said reagent is removed from the solution, all monomeres spontaneously join together to form the insoluble polymer.

The lipid forms 1/5th of the weight of the membrane and found entirely in the form of molecule known as phospholipid. Each phospholipid molecule is like a clothespin, the head bearing electrically charged atoms and the two legs are formed of long chain fatty acid. This molecule is insoluble in water but in structural combination which is known as micelle (a group of phospholipid molecules), it becomes soluble. The micelle is a stable structures which is formed by the combination of the phosphorus molecule and lipid through the hydrophobic bond. The behaviour of a phospholipid is determined by the properties of the micelle as a whole rather than by those of the individual molecule.

Thus we can say, that it is the combination of structural protein and phospholipid micelle that form the mitochondrial membrane. The membrane is a network consisting of alternating protein and micelle units linked by hydrophobic bonds. The two membranes, however, show different properties which clearly indicate that probably other components other than structural protein and micelle are responsible for these properties. Some scientists have also reported the presence of sulphur, iron, copper, vitamins and RNA in mitochondrial cortex, but the presence of RNA is very much doubted.

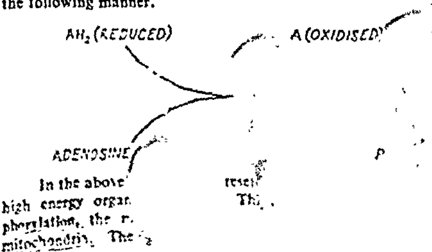
METABOLIC PATHWAY

The respiratory cycle involves the break down of carbohydrates, fats and proteins, so as to provide energy for the total organism. The whole process is completed in three phases. Under the phase I, the basic food stuffs are broken (glucose and ructose) down to simpler substances such as monosacchrides, glycerol, fatty acids and aminoacids. This, which is an exergonic reaction takes place in most multicellular animals inside the digestive tract. This phase is also referred as digestion. The digestion may be defined as the "process by which the food is converted into an assimilable form—into products which the individual cell can use for its

needs". The energy liberated by the chemical reactions involved is small, too small to be useful to the cell. It is simply dissipated in the form of heat. The phase II which occurs exclusively within the cell, involves further degreding the end products of hydrolysis, *i. e.* of digestion with the liberation of more energy and the production of three substances, *i.e.* acetyl coenzyme A, alpha-ketogluaric acid and oxaloacetic acid. When these ... ey generally enter into ... is the final common ... ie phase III. In the following pages we shall confine ourselves to the phase II and III.

PHASE II

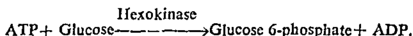
GLYCOLYSIS—Glucose oxidation liberates energy with the production of water and Co_2. But this involves a series of highly complicated interlocking steps. First of all glucose phosphorelated and finally changes into the three carbon compound, the pyruvic acid. In this process some small amount of energy is liberated, in the form of two high energy phosphate groups. These two high energy phosphate groups combine with the adenosine diphosphate (ADP) to form two molecules of ATP. The energy then is stored in the phosphate bonds of ATP or released as heat (during muscles contraction). During the glycolytic cycles, ATP donates high energy phosphate to another compound, thus enabling that compound to react chemically. Consequently, energy is both utilized and released in the cycle. The net gain being two molecules of ATP in the whole of the glycolysis. This can be represented in the following manner.



In the above ... resen ... high energy organ ... Th ... phorylation, the r ... mitochondria. The ...

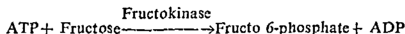
oxidation is coupled with phosphorylation. If these two events do not couple, then it results in the liberation of heat without concomitant ATP formation. The glycolytic cycle makes a very little contribution to the pool of ATP in the cell in comparsion to the later phase of respiration. The detailed out line reactions involved in the glycolytic reactions are as follows :—

Glucose phosphorylation—In the process, a compound is formed which contain glucose and phosphate. ATP generally enters into the reaction as follows.



The above reaction is catalysed by an enzyme **hexokinase.** This type of an enzyme is also called as **glucokinase.** ADP differs from ATP in having one less high energy phosphate bond. There is one more advantage of this phosphorylation. Glucose moves very easily through the plasma membrane where as the glucose 6-phosphate does not, thereby preventing the glucose from diffusing out of the cell. This glucose 6-phosphate can by hydrolized directely by some cells, specially liver cells by an enzyme **phosphatase** so as to produce again the glucose molecule. This positively occurs in the liver cells where it contitutes one of the mechanism for maintaing the blood sugar constant.

The glucose 6-phosphate is converted into fructose 6-phosphate by an enzyme **phosphohexa isomerase.** Fructose 6-phosphate can also arise directly from fructose.



It is next phosphorelated to form fructose 1, 6-diphosphate by the enzyme **phosphofructokinase** in the presence of Mg^{++} and ATP.

The next step involves the splitting of the fructose 1, 6-diphosphate by an enzyme **aldolase** into two triose monophosphates. The two monophosphate triose form an equilibrium mixture. Next 3-phosphoglyceric aldehyde is converted into 1,3—diphosphoglyceric

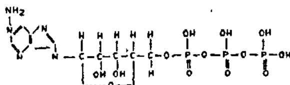


Fig. 46. Adenosine triphosphate (ATP).

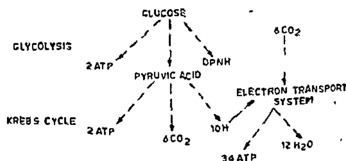


Fig. 47. Respiratory pathway in Mitochondria.

acid in the presence of a dehydragenase and coenzyme 1; 1,3 diphosphoglyceric aldehyde being a possible intermediate product.

It should be noted here that one of the phosphate in 1,3 diphosphoglyceric acid is of the high energy variety. The next step in the reaction involves the cleavage of this bond with the formation of 3—phosphoglyceric acid and the releasing of energy which combines either with ADP to form ATP or function to carried out the chemical activities or to produce the heat for mechanical activity.

The phosphoric acid is next transferred from 3 to 2 position by the enzyme phosphoglyceromutase so that 2-phosphoglyceric acid is formed. In the next step, the 2-phosphoglyceric acid is converted into the enol of 2-phosphopyruvic acid by the enzyme enalase. This molecule contains a high energy phosphate bond. 2-phosphopyruvic acid then losses its phosphoric acid releasing energy and forming pyruvic acid. In this way it is quite clear from chemical equations, where and how the two high energy phosphate groups are liberated.

It should be noted that in the entire process leading to the formation of pyruvic acid, no oxygen is used. It is because of this reason that the entire sequence is termed as an aerobic. When sufficie[illegible] O. [illegible] converted into acetyl coenzy[illegible] O. [illegible] O_2 is available [illegible] pyruvic acid and then to other products. This clearly indicates that if O_2 is available in plenty, the pyruvic acid continues into the metabolic mill for the final transformation to water and Co_2.

PHASE III

The phase III constitute the tricarboxylic acid cycle. This is also known as the citric acid cycle or the krebs cycle. In this phase, the closely related end products of phase II may be completely oxidized to Co_2 and H_2O. In the figure 48 it is quite clear that the acetyl Co A react with oxaloacetate to form citric acid and to liberate the coenzyme. The citric acid than undergoes several tansformations untill oxaloacetic acid is once again formed. These are, however, the major steps and in each step there are several interlocking reactions, all of them are catalyzed by so many specific enzymes.

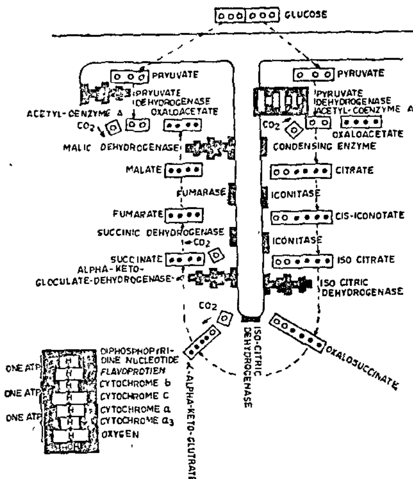


Fig. 48. Diagram showing the anaerobic and aerobic respiratory mitochondrial pathway.

It should be noted here that in the cycle only the 'acetyl' part of acetyl CoA is used up and as long as this component will enter into the cycle the reaction will continue.

Not only this cycle will continue in the presence of acetyl coenzyme A and oxygen but this will also continue even in the presenee of the other products of phase II metabolism, the alpha keto glutaric acids and oxaloacetic acid. Because all the three end products can take part, the tricarboxylic acid cycle is truely the final common metabolic pathway.

Enzymes related to metabolic pathway—However, the number of enzymes taking part in the degradiation of the foodstuff through the three phases of metabolism is great. As such it will be troblesome to take every one here, but they may be considered as belonging to various catagories.

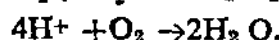
(1) Hydrolases.
(2) Oxydases.
(3) Dehydrogenase.
(4) Transferase.
(5) Isomerase.
(6) Decarboxylase.

(1) **Hydrolases**—These enzymes catalyze the reaction in which the water splits the complex organic molecules into simpler compounds.

(2) **Oxidases**—Broadly speaking, the oxidase is an enzyme that promotes the oxidation reaction. More specifically, after the oxidation reaction has been completed as a result of the presence of an oxidase, the reduced O_2 appears in the form of peroxide or water, as.



$$H_2 + O_2 \longrightarrow H_2O_2$$



$$4H^+ + O_2 \rightarrow 2H_2O.$$

For example, the cytochrome concern to this group. The cytochromes are found in most cell, but they are particularly concentrated in muscle cells, which suggests that cytochromes are essential for reaction which must proceed rapidly and with a sudden burst of energy. Chemically, the cytochrome is a cell pigment, containing iron and protein. There are also certain oxidases which are copper containing proteins.

(3) **Dehydrogenase**—The dehydrogenase indicates that these enzymes concern with the electron transfer in various stages in the

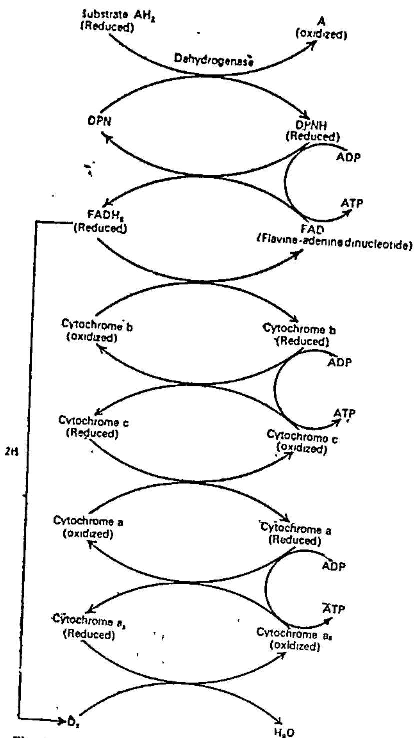


Fig. 49. The complex scheme of electron transport system.

process of dehydrogenation. Most organic compounds are oxidised in this way.

Nicotinamide-adenine dinucleotide (NAD), coenzyme I and Nicotinamide adenine dinucleotide phosphate, Coenzyme II, are synthesized by many cell. *It* is to be noted here, that **NDA** was formerly referred as diphosphopyridine nucleotides (DPN), and **NADP** was known as triphosphopyridine nucleotide **TPN**. Here we have used DPN and **TPN**. These pyridine nucleotides are coenzymes which function quite commonly with the flavin containing enzymes, the so called flavoprotein. This flavoprotein dinucleotide is essential component of these enzymes and therefore abbreviation **FAD** is used.

The cytochromes are not believed to act directly on the substrate. This is, apparently the role of the dehydrogenase. In a typical metabolic respiratory pathway (fig-49), the substrate i oxidized. At the same time DPN is reduced. DPNH now reac with flavoprotein to be oxidised while the flavoprotein is reduce Further, the two molecules of cytochrome *b* are reduced with t liberation of two hydrogen ions. The electrons are then handed down to cytochrome chain untill finally molecular oxygen is reduced to oxygen ion which reacts with the two hydrogen ions to form water. In this chain however, the energy is liberated.

It is quite clear from the figure-49 that there can be a continuous cycling at each point. For example, the DPN is being reduced by accepting electrons, it is also being reformed from the reduced state, in the process of which the electrons are given up. So long as there is an adequate store of reduced substrate at one end of the chain, and an adequate supply of oxygen, or other electron acepter at the other end, the wheel will turn so to speak. The potential gradient represented at the system not only provide the energy to turn the wheels of the respiratory chain, but it also provides energy at three points in the system which is, however, utilized to drive the adenosine diphosphate (ADP)+ inorganic phosphate so as to form the high energy adenosine triphosphate (ATP). ATP is like a charged storage battery, its energy is on cell for cellular processes.

Transferase—These enzymes act to catalyze the transfer to a radical from one compound to another. Radicals capable of being transferred include methyl phosphate and amide group.

Isomerases—There are enzymes which catalyze internal changes

in a molecule. For example as the conversion of glucose-6-phosphate to fructose-6-phosphate and glucose-1-phosphate to fructose 6-phosphate.

Decarboxylases—Carbon-dioxide however, can be removed from themolecule of carboxylic acid without oxidation, with the help of these enzymes.

FUNCTIONS OF MITOCHONDRIA

There can't be two openion for the fact that the main mitochondrial function is to generate high energy ATP. But the question ar [illegible] w this er [illegible] site of ac [illegible] fter the ex [illegible] 54) and Albert Lehninger (1960) much of the mystery as to how the mitochondria generate the energy has been revealed. The following account is based on their researches.

The role of respiratory chains in the formation of high-energy ATP has already been discussed in the chapter of "Cell metabolism". It has also been pointed out that mitochondria are capable enough of producing these molecules of ATP for each pair of electron released by oxidation.

Role of mitochondrial particles—The particles which are found anchored on the outer surface of the outer membrane and inner surface of the inner membrane are essential for this process. They do three main functions :—

1. Carrying out the oxidation reaction that supply the electrons.
2. Catalyzing synthetic reactions which are powered by ATP.
3. Transfering the electrons along a chain of complexes that synthesize ATP.

The available literature indicate that the particles located on the outer membrane perform the functions 1 and 2 whereas the function 3 is performed by the particles located on the inner membrane.

Green and his co-workers suggested that the outer particles provide electrons by the oxidation of fatty acid or from the krebs cycle. In the latter case succinate results where as in the former the electron is accepted by the coenzyme DPN (diphosphopyridine) which is thereby reduced to DPNH. Succinate and DPNH carry

the electrons from outer particles across the space between the two membranes and finally across the inner membrane so as to hand over them to the inner particles. It is in these inner particles that the electrons move along a respiratory chain, producing thereby three molecules of ATP.

Electron-transport system—The electron transfer chains are believed to be arranged in four groups or complexes. These complexes are stationary and are separated from one another by the lipid. They lie in the inner particle. The complex I and II lies in the base of the particle, complex III lies in the stalk and complex IV lies in the knob of the particle. The chain starts with complex I if the electrons come from DPNH; it starts with complex II if the

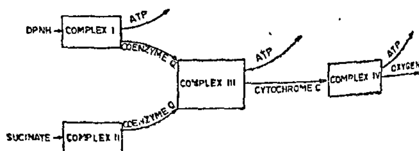


Fig. 50. Electron transmission through a series of complxes inside the mitochondria

electrons are donated by succinate, a product of citric acid cycle. In both cases, the electrons travel on from complex I and II to the complex III and then to the complex IV. At the end of the chain, the electrons are carried off by a molecule of oxygen. At each st molecule of A olecule by the complex of the stalk and one molecule by the complex of the knob.

From complex I or II, the electrons suttled across a lipid layer to complex III by a catalyst, known as the co-enzyme. From complex III to IV, the electrons are carried by the cytochrome C. But how these electrons move within the complex? Bock and Criddle gave an ingenious explanation. They picture electron transfer occurring between sewinging groups of atoms. According to them each complex is thought to comprise 4-6 protein molecules; with their bases attached to the wall of the inner particle and their free ends projecting into the phospholipid core. Thus they appear just like pendulum whose free ends act as the electron

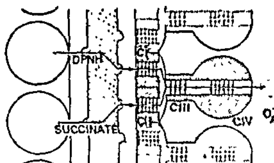


Fig. 51. Hypothetical arrangement of the four energy generating complexes within the electron-transfer particles. According to this, the complex I and II lie in the base of the particle; complex III in the stalk and complex IV in the knob.

acceptor. Bock and Criddle suggested that the electrons are handed over from one pendulum to other in the series as the pendulum swing back and forth. But why they swing and how the electron transfer occurs are yet to be explained in details.

ATP Production—Though the mechanism by which ATP is produced has already been discussed in the chapter of "Cell metabolism" yet it has been thought proper to reemphasis it here in the light of the specific role of mitochondria. To cut short, it involves the union of phosphorus by a high energy bond with ADP.

As the electrons move from one complex to the next, there is a drop in potential and thus an energy change. It has been argued that in mitochondria, as the electrons "coast" down hill there energy is used to form high energy bonds in ATP. The energy is passed to ATP from the electrons. It should be noted that all the energy does not end in ATP. Approximately 60% of the energy is utilized for the chemical interactions involved in the respiratory chain, only 40 percent thus end up in the form of ATP high energy bonds. This also explains as to why all living cells require an exogenous source of energy.

G. C. Webster studied the detail, of the process in complex IV and it is assumed that similar steps occur in other complexes also. According to him, reduced cytochrome C becomes joined by a high energy bond to a copper containing group which is found in the protein molecule of complex IV. This results in the formation of a new compound which interact with another protein

molecule—a so called coupling factor. In other words, the coupling factor replaces complex IV, leaving the high energy bond existing between cytochrome C and the coupling factor. In a similar way cytochrome C is replaced by an inorganic phosphate group; as a result of this the coupling factor and the phosphate group become united by a high energy bond. Now the coupling factor is replaced by ADP. This results in the formation of ATP.

1. Cytochrome c+complex IV→cytochrome c~complex IV
2. Cytochrome c~complex IV+coupling factor→
   cytochrome c~coupling factor+complex IV
3. Cytochrome c~coupling factor+P→
   coupling factor~P+cytochrome c
4. Coupling factor~P+ADP→ADP~P (ATP+
   coupling factor~high energy bond.

Transportation of ATP molecules—*High energy ATP molecules remain concentrated with in the mitochondria. How these molecules come out of the organelle or how these molecules move from mitochondria to the site where the energy is required. The mitochondria can move by virtue of changes in the dimensions of their membranes It has been observed in the kidney tubular cells, that they become concentrated at a site in the cell where energy demands are high This change in the dimensions of the membrane not only provides movement to the organelle but also responsible for other activities as well. It has been argued that when the membrane contracts the internal hydrostatic pressure rises : this creates a transmembrane gradient with the result* H_2O *and ATP are squeezed out of the organelle. When they have squeezed, the internal concentration of ATP decreases, the membrane relax and thus the cycle is ready to begin again. In short this all can be looked upon as a self regulating, feed back mechanism.*

Control of mitochondrial function—The respiratory mechanism of mitochondria is controlled by several factors. The influence of ATP on mitochondrion membrane does play a role but this is only a very small part of what actually involves very complex and interrelated mechanisms. Further mitochondria themselves are capable of adjusting their respiratory rate to the availability of inorganic phosphate and ADP. This is said to be the result of compulsory coupling or coupled phosphorylation or oxidative phosphorylation So long as there is a compulsory coupling any thing that regulates the supply of either inorganic phosphate or ADP will control the

respiration. It has been pointed out that when these ingredients are present in excessive supply, mitochondrial respiration is still regulated in accordance to cellular demands of energy. Ernster (1965) illustrated the abnormal concentration of mitochondria in the cells of his one of the patient's skeletal muscles, yet there was a complete lack of respiratory control. The question arises why this over production of mitochondria and why respiratory control was lost. *These are still unanswered questions.*

The respiratory mechanism of mitochondria is regulated by internal as well as external factors. Two known external factors, *i. e.* thyroid and parathyroid hormones are known but how they influence the mitochondrial function is not fully explained uptill now.

SUMMARY

Mitochondria, the power house of the cell are sausage-shaped structures which possess an average diameter of $0{\cdot}5\mu$ and a length of about $1{\cdot}5\mu$. Their sizes and number are variable in a cell. Each mitochondrion is enveloped in a double membranous envelop, the each membrane being a typical unit membrane about 75A° thick. Their inner membrane forms elongated sacs called cristae which extend into the interior of the mitochondrion. The space between the two membranes contains a fluid rich in coenzymes. Its interior has generally a homogeneous matrix. The surface of both the membranes are very much sprinkled with thousands of very small particles which are about 100A° in diameter. The inner membrane particles are formed of a base,stalk and head. Chemically each membrane is formed of proteins and lipids.

The function of the mitochondria is to produce the high-energy ATP. It produces ATP by a complex procedure in which pairs of electrons are passed along four the outer-membrane particles to the inner-membrane particles ; in the latter they pass along a series of four complexes. The outer-membrane particles provide electrons by oxidation of fatty acids or from the krebs cycle. DPNH and succinate carry the electrons from outer to inner-membrane particles. In the inner particles they pass along a respiratory chain to produce 3 molecules of ATP for each pair of electrons.

To transport the ATP to a site of action, the mitochondria, it is believed, move and become concentrated at a site in the cell. Their movement is caused because of change in the dimensions of their membrane. However it is yet to be confirmed. The contraction of the membrane also causes the inner substances (ATP) to squeeze out.

The respiratory mechanism of the mitochondria is regulated both by external and internal factors. Thyroid and parathyroid are the known external factors but how they influence the mitochondrial function is not fully known.

9

LYSOSOMES

The lysosomes (*lyso*=digestive ; *some*=body) represent a new discovery among the cytoplasmic particles These are present in most of the animal cells and a few plant cells. For long they were called **pericanalicular dense bodies**, suggesting their location but not their function. In 1955 **Christian de Duve** renamed these bodies as lysosomes since they contained hydrolytic enzymes in them. In fact lysosome is a lytic body. Upon examination under the electron microscope, these bodies were found to comprise membrane bounded structures whose appearance is markedly affected by the osmotic conditions present during preparation.

Origin—There are several probabilities as regards their origin. It has been suggested that they have a multiple origin, depending upon the tissue in which they are located or their function in a cell. A lysosome may represent a pinocytic vacuole, implying **extracellular origin.** Its enzymatic activities may then develop after it has become a part of the cytoplasmic machinery. There are also evidences to suggest that it has **originated from the Golgi complex** and represent zymogen granules. It is very difficult to ascertain or to establish their origin, because of their variability in shape, size and density from cell to cell, but it appears that in all probability the membrane appears to have originated from Golgi complex and the enzymes from ribosomal activity. Recently it has been suggested that in the outer part of a series of vesicles of Golgi complex, the lysosomal enzymes are packaged into organelles surrounded by single lipoprotein membrane. These "nascent granules" develop into "primary lysosomes" in which the enzymes are stored in an inactive form, ready for use.

Distribution—The distribution of lysosomes, though not fully worked out, yet has been observed in the cells of liver, spleen, brain, thyroid of mammals, the meristematic cells of plants and in some protozoans. It is very likely they occur in all the cells. In white blood corpuscles, they are large and numerous. This suggests that they are large and numerous in cells with digestive functions.

Morphology—They usually appear as a dense body, surrounded by a membrane; normally they vary in size from 0·4μ to 0·8μ in diameter, but they may be as large as 5μ in mammalian kidney and are exceedingly large in phagocytes. It may be spherical, irregular, or rod-like in shape, being covered by a single layered membrane composed of lipoprotein. The internal organisation is quite variable due to their different functional activities. Some lysosomes are uniformally solid, others have a very dense outer zone with a less dense core, and still others possess cavities or vacuoles with granular material. Their contents are denser than mitochondria. They are not constant in their morphology.

Kinds—Two types of lysosomes have been reported, *i. e.* the digestive vacuole and the residual bodies. The digestive vacuole in the rat kidney cell is formed due to the fusion of the original lysosome body with a vacuole containing substances ingested by the cell. The residual bodies comprise myelin figures, representing substances that have not been digested, such as fat.

Chemistry of Lysosome—Lysosomes contain variety of enzymes (acid [illegible] ease, phosphatases, cath[illegible] down all the major cons[illegible] ins, carbohydrates, fats, [illegible] mes function more efficiently under slightly acid condition as such they are collectively called as acid hydrolases. Like other proteins, these enzymes are synthesized in ribosomes with in the folded membrane of endoplasmic reticulum. The simplest chemical definition of a lysosome is "a body rich in acid hydrolase." High levels of acid phosphatase have been found in a number of different tissue, including liver, kidney, endocrine glands, plant roots, and fungi. It was found in the cells of these tissues that acid phosphatase activity is particularly conspicuous in the Golgi complex and is associated with lysosomes. Lysosomes do not possess oxidative enzymes: a property which [illegible] This also suggests that [illegible] mic particles. When the membrane of lysosome is disrupted, all the enzymes contained are released and simultaneously become active. As long as the membrane is intact, the enzymes are inactive.

The lysosomes should not be confused with other similar particles found distributed in the cytoplasm. These particles have different enzyme contents. They are normally high in uricase and

this uricase activity exhibited even when their membranes are intact. Some lysosomes (*e. g.* liver) contain ferritin granules which are wanting in uricase particles. Some scientists believe that the uricase particles and lysosomes are identical except that the former is insoluble portion of the latter.

Homology of Lysosome—An interesting homology lies between the lysosomes and the acrosome of a sperm. An acrosome shows the acid phosphatase activity, so may be referred as the specialised lysosome. This fact, however, points out towards the origin of lysosome. The acrosome always originate from the Golgi complex whereas some lysosomes are also derived from the complex. Further [illegible] in [illegible] of fertilization.

FUNCTIONS

1. Lysosomal digestion of External particles—Lysosomes also act as agency through which certain kinds of materials get access to cells. Foreign proteins and other materials undergo digestion within the cell as a result of pinocytosis and phagocytosis (collectively called endocytosis) Ingested particles become enclosed in membrane, derived from plasma membrane to form vacuole which are called phagosomes. The phagosome moves towards the lysosome. They come close to each other, their membranes fuse and some how they then form one body. In this body the lysosomal enzymes begin

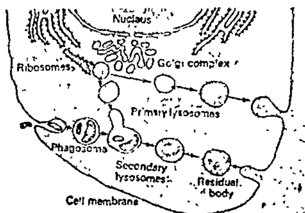


Fig. 52. Diagram depicting the function of lysosomes—the intra-cellular digestion.

the process of digestion of the foreign material that has come contained in phagosome. After the enzymatic digestion, the digested material diffuses out into the hyaloplasm of the cell. The undigestable materials remain segregated from the cytoplasm within "residual bodies" which may remain in the cell for a long time and then it moves on to the cell membrane where the so called reverse phagocytosis occurs, thereby excreting the contents of the residual body.

The devouring of bacterium by the white blood corpuscles has been studied and it was found that in the process, the lysosomes play the major role. As soon as the bacterium is engulfed by the cell the lysosomes ... leasing their ... ith the help ... the process, the lysosomes are lost and the corpuscles also die ultimately, having carried out major function in the body.

2. ... sosomal enzymes ... haps by reverse p... ed outside the cell where they then digest the structure concerned. This mechanism also provides an explanation that how sperm penetrates the ovum and that how osteoclasts (cells that destroy bone) function.

3. **Cellular digestion**—There are cells in the body which are short lived, *e. g.* outer layer of skin and the mucous membrane linings of the body. The cells are continually being replaced, and ... anism by which ... cells rupture, the ... n the whole cell is digested. This digestive process which occurs quite rapidly in dead cells is called autolysis. Thus the process of tissue degeneration or necrosis can be attributed in part to this lysosomal activity.

4. **Digestion of Intra-cellular structures**—A specialized form

cell's substance can be broken down so as to provide energy or material needed to maintain the life of cell Presumably for this method an unneeded elements are broken down Proteins, fats and polysaccharides can be synthesized and stored in various forms in the cell. During starvation, these substances are digested to provide energy that normally comes from ingested food. The large vacuoles containing enzymes move to the region where lysosomes lie. These lysosomes increase in number and show acid phosphatase activity. Thus autophagy provides an intra-cellular digestive mechanism.

5. **As trigger of cell division**—It has been observed that the dividing cells have comparatively few lysosomes and that too lie on the periphery of the cell instead of near the nucleus. This suggests that the break down of lysosomes may act as a trigger for mitosis in cells which are prepared for it. Allison (1967) and others have suggested that mitosis is ordinarily inhibited by some kind of "repressor" substance and the lysosomal mechanism is involved in depressing it. It has also been observed that the animal egg cell does not normally divide unless it is stimulated to do so. Some cortical "granules" have been reported in the outer part of the egg which contains same enzymes and staining reaction as lysosomes. The acrosome enzymes, besides facilitating the penetration of sperm need into the egg, also seem to initiate the disruption of the cortica granules.

6. **As causative agent of certain diseases**—There are evidences to suggest that the lysosomes are involved in causing some diseases. In most of such cases, it was found that untimely rupture of the lysosomal membrane lead to some form of cell damage. The enzyme, DNAase has been reported to be present in lysosome which when released, the chromosomes break and arrangement appears. It is not yet definite that lysosomal DNAase can enter nuclei and can break chromosomes in living cells ; but there are strong indications in this direction. According to Allison (1967), the chromosomal changes because of lysosomal induction may help to explain the origin of certain types of cancer.

SUMMARY

Inside the cell, also found certain bodies containing digestive enzymes capable of lysis. These are called lysosomes. Formerly these were regarded as pericanalicular dense bodies. Their shape varies and so their sizes.

Normally they vary from $0{\cdot}4\mu$ to $0\ 8\mu$ in diameter. Most of them are round, but rod-like and irregular shaped bodies are also met. They are bounded by membrane, formed of lipo-protein. It has been suggested that lysosomes have originated form the Golgi complex. Extra-cellular origin has also been reported. Mostly they are formed in association of the Golgi complex or ER.

Lysosomes contain variety of enzymes which function more efficiently under slightly acidic conditions, as such they are collectively called as acid hydroleses. The more important enzymes includes ribonuclease, deoxyribo-nuclease, phosphatases, sulfatases, cathepsins, .

Lysosomes have atleast four functions, *i. e.* the diges-tion of large particles that enter the cell, the digestion of intracellular substances, the digestion of the cell it self and the digestion of substances external to 'the cell.

10

PLASTIDS

These are the protoplasmic organoids, intimately related to the metabolic proceses of plant cells and tissues. They found throughout the plant kingdom except possibly in bacteria, certain algae, myxomycetes and fungi. They are characterised by the presence of pigments. They are however also found in few animal cells, notably in some flegellate protozoans. These bodies play an important role in different biological metabolism. They confront the biologists with three kinds of problems, *i. e.* cytological, biochemical, and phylogenetic Cytologists have learnt much about the plastids, but however, there are some important points regarding their structure, visible alterations and relations to other cytoplasmic differentiations which still need investigations. Biochemists are gradually approaching a better understanding of the exact chemical changes which take place in plastids during photosynthesis. They are also busy in studying the significant relation between pigments and the vitamins. Systematists are trying to know more about the historical origin of plastids and their role in divergent evolution of organism with different types of nutrition.

Classification—The classification of plastids is mainly based upon the colour or pigment contents. These are of three kinds :—

(a) **Leucoplasts**—These are the plastids which are devoid of pigments and membranous structures distinguishing the chromoplasts. They are meant mainly for storing the starch, oil, and proteins.

(b) **Chromoplasts**—These are the pigmented plastid containing colouring matter in them. They are found in the cells of leaves, many flowers, fruits, etc. The familiar chromoplast is the chloroplast or the green plastid containing chlorophyll pigment which is instrumental in initiating the process of photosynthesis.

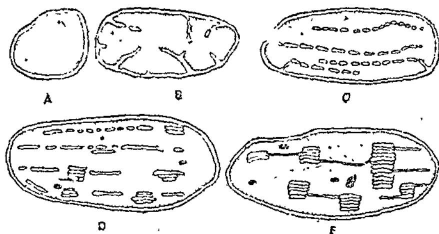
(c) **Chromatophores**—In the cells of blue-green algae the pigments are not organized within a descrete plastid body but are often arranged on lamellar structures in concentric rings or plates. As such here the term chromatophore is used instead of plastid,

TABLE 3—SHOWING MAJOR PLASTIDS OF THE PLANTS

Type	Occurrence	Functions	Major pigments
(A) CHROMOPLAST			
1. Chloroplast	Higher plants and certain algae.	Photosynthesis.	Chlorophyll a and b
3. Phaecoplast	Brown algae ; diatoms, dinoflagellates, etc.	Light absorption.	Fucoxanthin.
3. Rhodoplast	Red algae.	Light absoption.	Phycoerythrin.
(B) CHROMATOPHORES			
1. Blue-green	Blue green algae.	Photosynthesis.	Phycocyanin ; phycoerythrin.
2. Bacterial	Purple sulphur bacteria ; Green sulphur bacteria.	Absorbs infarred region.	Bacteriochlorophyll ; bacteriorviridin.
3. Carotenoid	Tomato ; red pepper ; flower parts ; fungi ; bacteria.		Lycopene ; capsanthin.
(C) LEUCOPLAST			
1. Amyloplast	Food storage cells.	Starch storage	None.
2. Elaioplast	Certain monocotyledons.	Oil storage	None.
3. Proteinoplast (Aleuronplast)	Seeds (Brazil-nut)	Protein storage	None.

Origin and development—Most authors believe that plastids always arise from pre-existing ones. It has been noted that they show no dividing properties like chromosomes at the time of division but they are transmitted as usual directly in the daughter cells. Thus a kind of genetic continuity is maintained in them from generation to generation.

According to recent studies, the **proplastid** is the dividing element rather than the mature plastid. It is regarded as "stem plastid" which give rise to leucoplasts or immature lamellar plastids. These later on develop into chromoplasts of specific kind or proteinoplasts. In ceratin monocotyledons, elaioplasts develop from old chloroplasts which loose their chlorophyll and accumulate oil. The developmental pathway is controlled by the external factors like light and heat and internal factors as reside in cytoplasm. and



Fig, 53. Development of a chromoplast in the light. A—proplastid; B—vesicle formation; C—differentiation, development of *colour*; *D—differentiation, development of lamellae*; E—maturation.

nucleus. At present we possess no evidence that plastids arise *de novo*; they arise from the element already present in the cytoplasm. It is not clear that how proplastids originate but their development from proplastids to mature plastids has been studied quite extensively.

During the development, the proplastid first increases in size and the inner limiting boundary invaginates at several places, producing small vesicle-like structures. These vesicles later on pinch

off as free units. Then they are grouped in several arrgangements to form the lamellae of mature plastids. This all takes place in the presence of light. In the absence of light, there is a different pathway of the plastid development and maturation. In night or dark, the lamellar pattern always appears as a clustures of discs, a crystal lattice or in the form of a series of concentric rings.

Here we shall consider the chloroplast, as they are most common of all the plastids and are of great biological importance.

CHLOROPLAST

Morphology—It is one of the largest cytoplasmic structures which can be well observed under the simple microscope. The size, shape and distribution of chloroplasts vary in different cells and species, but they are relatively constant within the same tissue' The average size varies from 4 to 6 μ in diameter and 1 to 3 μ in thickness. The diameter is rather constant for a given cell type but sexual and genetic differences may be found. The shape too vary considerably. They may be spherical, ovoidal or discoidal. In certain cells they have special shapes. They are sometimes club-shaped. Algae often possess a single huge chloroplast that appear as a network, a spiral, band, or a stellate plate. They are sometimes homogeneously distributed within the cytoplasm, but are seldom packed near the nucleus or close to the cell wall. Their

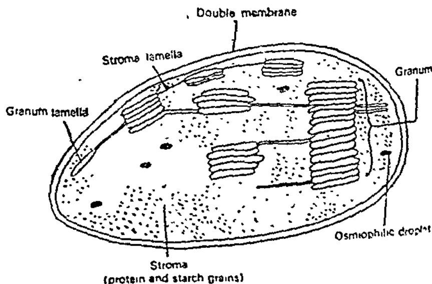


Fig. 51. Submicroscopic structure of mature chloroplast.

distribution depends largely on external conditions such as light intensity. Their number is relatively kept constant in the different plants. It has been indicated by Haberlandt (1914) that they are about 400,000 per square millimeter in the leaf of *Ricinus communis.*

Structure—The fully developed chloroplast is limited by a semi-permeable membrane. The membrane actually comprise two separate layers, each being 40 to 60 A° thick and the space between them vary from 25 to 75 A°. Inside is filled with a proteinaceous matrix called stroma. The stroma contains starch grains and osmiophilic droplets. The osmiophilic droplets increase in number with the increase in growth of chloroplast or when it becomes inactive. Numerous small platelets, the grana (sig. granum) found embedded in stroma. Their number, however, vary in chloroplast. The mesophyll cell of spinach has 40 to 60 grana per chloroplast where as one granum per plastid is found in *Egulena.* Each granum consists of double membrane discs or lamellae which vary in thickness and are of two types, *i.e.* granum lamellae and stroma lamellae.

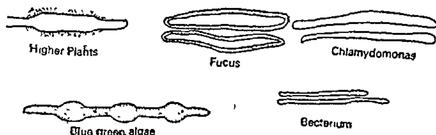


Fig. 55. Different types of lamellar organisation.

The arrangement of lamellae varies with the type of plants. These variations have been shown as differences in the sizes of the lamellae and the degree of thickening along the membranes. Chemical tests show that granum consists of mainly the proteins and lipids. There are no grana in *Chlamydomonas.*

In *Chlamydomonas* the chloroplast (one in cell) envelop is continuous with the elements of the endoplasmic reticulum. In *Ochromonas danica* (one in a cell) there is present an extra envelop outside the limiting membrane. This envelop is continuous with nuclear membrane but is not porous. Such extra envelop has not been observed in Rhodophyta or Chlorophyta.

Physiochemical properties of chloroplasts

1. They have greater resistance to osmotic action and fixation. This distinguishes them from mitochondria and other plastids.

2. They have a strong reducing effect. Very easily they can reduce silver nitrate even in dark. This property is related to photosynthesis.

3. They have a higher specific weight than cytoplasm.

4. They can also act as a thixotropic gel, undergoing liquefication under various mechanical actions.

Chemistry—The chemical composition of chloroplast suggests that it mainly comprises the proteins and lipids, after the extraction of chlorophyll. Attempts have bave been made to ascertain the exact form and asssociation between these materials and the chlorophyll. The results have led to a hyphothesis of chloroplast structure. According to this hypothesis, the chlorophyll forms a series of monomolecular films on the surface of numerous protein layers, lying more or less parallel throughout the granum. The

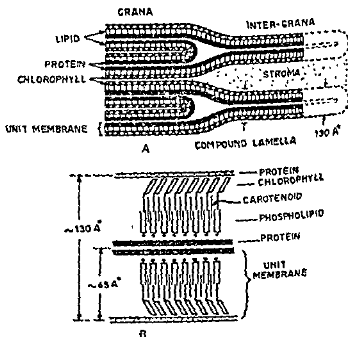


Fig. 56 Showing the detail of chlorophyll organisation, Hypothetical representation of lamellation in the mosophyll chloroplast of corn. A—density observed in sections of osmium fix material; B—an interpretation of this in terms of protein, lipid and chlorphyll.

chlorophyll molecules have their hydrophilic ends associated with the protein and their lipophilic ends with lipid molecules. This arrangement is so set that in between each two protein layers there are two films of chlorophyll molecules, a double layer of lipid molecules, a few xanthophyll molecules and water. The distance between the two protein layers is about 0·005 μ. To what extent this hypothesis is valid cannot be said but this is valuable as long as it is subjected to experimental tests.

In some algae, the chloroplast also contain peculiar bodies, the pyrenoids. They appear like small masses of protein in the stroma. They play an important role in the elaboration or deposition of carbohydrates as starch grains develop in their immediate vicinity and form a dense mass about each of them.

TABLE 4—SHOWING THE CHEMICAL COMPOSITION OF THE CHLOROPLAST

Substance	Precentage (by dry weight)	Substance	Percentage (by dry weight
Protein	30—55	RNA	2—3
lipid	20—30	DNA	0·5
Chlrophyll (75% a, 25% b)	9	Cytochrome f	0·1
		Vitamina K	0·004
(Carotenoids (75% xantho-phyll, 25% carotene)	4·5	Vitamin E	0·08
		Mg, Fe, Cu, Mn, Zn, P	Traces

As indicated in the above table that the protein contents represent not only the protein of the membranes but also of the several enzymes within the chloroplast. The different enzymes present in chloroplast vary according to the level of maturity of the plastid. For example certain enzymes have been isolated from proplastid which, however, help in the synthesis of proteins, lipids and RNA. As the plastid matures, more enzymes are formed, including those necessary for the synthesis of chlorphyll, carotenoids or other pigments and for the synthesis of carbohydrate. A mature plastid also possesses several enzymes for the photosynthetic activity and synthesis of proteins.

Functions :

1. The main function of chloroplast is to take active part in photosynthesis. Quanta of sun energy is trapped by the chlorophyll in the chloroplast during photosynthesis, and is then employed in combination of carbon dioxide and water to form carbohydrates with the loss of oxygen.

2. The similarities which chloroplast has with the mitochondria as regards structure and function, led to believe that they contain the enzymes for the Krebs cycle and for the synthesis of fatty acids. Chloroplast actively incorporates aminoacids in the presence of ATP and are capable of a certain degree of protein synthesis.

SUMMARY

Plastids are microscopic organelles found in plant cells such as **leucoplasts**, in which the starch granules develop, the **chloroplast**, which contain the chlorophyll (green pigment) of the plants, and the **chromoplasts**, which however contain the other pigments. Best known are the chloroplasts, since they have been of interest in the extensive studies on photosynthesis. Chloroplast originate from minute submicroscopic ameboid proplastids. They are of various shapes such as spherical, discoidal or ovoidal and sometimes even disck-shaped. The discoidal ones frequently have a colourless centre containing the starch granules. There are two important functions of the chloroplast. First, they take part in active photosynthetic activities and secondly they actively incorporates aminoacids in the presence of ATP and are capable of to a certain degree of protein synthesis.

11

CILIA, FLAGELLA AND BASAL BODIES

CILIA AND FLAGELLA

Cilia and flagella are extremely delicate filamentous structures, mostly external to the cell. They always arise from the cytoplasm and make their physical connection with the plasma membrane. They, though not found in all the cells, yet are widespread among plant and animal cells. Among protozoans, these organelles are quite common and are usually used for locomotion. In the plants they are restricted to the unicellular algae and certain reproductive cells of higher forms.

The cilia and flagella differ from each other, mainly in their number and size. Cilia are generally shorter than the flagella. The cilia may be 5μ to 10μ in length while the flagella are upto 150μ in length. The most common number of the flagella are one or two, although they r [illegible] to as many as 1[illegible] generally exhibit a sweeping or pendular movements and the several cilia present are often co-ordinated in th[illegible] T[illegible] flagella [illegible] f one [illegible]. They are morphologically similar, so much so that the two terms are often used interchangeable. The account given below is essentially applicable to both except otherwise mentioned.

Structure— Though as early as 1887 Jansen described the structure of the sperm flagellum but the detailed structure of the flagellum of sperm was given by Hodge in 1950. Manton described the structure of the cilia of higher plants. Both the workers reached more or less on the same conclusions as regards their fine structure; however, there are certain variations in the structural details of different organisms.

In each case organelles consist of eleven submicrosocopic, fibrillae, oriented along the axis. Out of these eleven filaments or fibres, nine are double and are situated at the periphery; the two are central and are single. This bundle of filaments is embedded

a cytoplas ... enclosed in a double me ... membrane. ... membrane being about 40A° thick and the two membranes are 30 A° apart. Each of the nine peripheral filaments or fibrils, is composed of two halves, each 180 to 250A° in diameter. The two halves are surrounded by a wall and are separated from each other by the continuation of the same wall with a thickness of 45 A°. Several workers have confirmed the presence of two arms on one of the two members of the doublet in each of the nine outer fibrils. These arms are all oriented in the same direction around the cylinder. Each arm is about 50 A° thick and 150 A° long. The two central fibrils are enclosed in a common sheath within which they are completely separated. These component fibrils run longitudinally within the shaft and may extend into the cytoplasm below the cell surface.

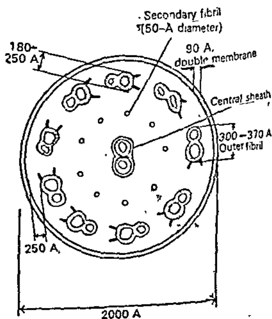


Fig. 57. Cross section of a flagellum (Diagrammatic)

The recent studies by Gibbons and Grimstone (1960) on *Pseudotrichonympha* and other flagellates have shown that the submicroscopic morphology of a flagellum varies according to the level of the flagellum. According to them in the distal portion of the flagellum few more fibrils are present in between the central and peripheral fibrils. These secondary fibrils are relatively smaller in size and diameter. Usually there are nine secondary fibrils, each with a diameter of 50 A°. In L.S. the secondary fibrils appear to be not perfectly straight but somewhat wavy or irregular. Further they suggested that at the distal end or tip of the flagellum, there is a tapering off or reduction of fibre structure. They studied the flagella of some

protozoans. According to them the arms of the outer fibrils disappear first and then the double nature of these fibre is lost. This arrangement, however, differs from one organism to another.

BASAL BODIES

The cilium or flagellum may be connected with cytoplasmic particle embedded in a clear layer of cytoplasm just beneath the plasma membrane. To this cytoplasmic particle several names have been given in a variety of plant and animal cells ; such as **kinetosomes, blepharoplasts, basal granules** and **basal bodies.** The most widely accepted term at present is the basal body.

The basal bodies are arranged uniformly in parallel rows under the cell surface. The basic pattern of fibril composition and arrangement in a basal body is somewhat different from that in a cilium or flagellum. The nine peripheral fibrils are composed of three units (triplet) rather than two, as found in the distal portion of the organelle. T ... the doub ... ody prop ... ary fibril ... ter fibers of the basal body often show fine, fiber-like connections between adjacent triplets as well as between each triplet and a single central fibril. This fine connections among the fibril component make up a pattern resembling a cart wheel. The central fibril is of 250 A° diameter.

Zone of transition— The region near the surface of the cells within which the external portion of the organelle is associated

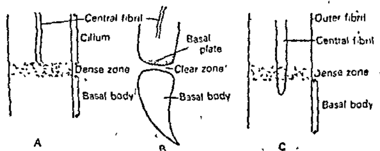


Fig. 58. Relations between a cilium and a basal body in mammals and protozoans (A), in molluscs and amphibians (B), and in *Tetrahymena* (C).

with the basal body is usually called as zone of transition There can be three variations in this zone in different animals.

1. In mammals and protozoans there is *no definite separation* between the cilium and the basal body. *However*, all the fibers of cilium do not continue into the basal body ; central fibers usually end above its upper surface, which is represented by a dense zone.

2. In molluscs and amphibians, the cilium and basal body are *completely* separated in the transition zone by a basal plate. The basal plate is continuous with the outer fibers of cilium. In this case, a clear zone lies beneath the basal plate and above the basal body.

3. In *Tetrahymena*, the two are completely separated and at the zone of transition lies a dense region.

The metazoans with the exception of mammals possess rootlets extending from the basal bodies further into the cytoplasm. These are most common in ciliated epithelial cells. The number of rootlets



Fig. 59. Basal bodies with double rootlets (A) as in some molluscs and with one rootlet (B) as in *Rana* (frog.)

vary but their number is *constant for a species*. They (rootlets) may have cross striations 60 to 100 m μ wide. Between the major cross striations, the space varies from 550A° to 700A°, and in this narrower bands may be present. In some cases in addition to rootlets, short knobs may project from one side of the basal body.

<u>Centrioles</u>—The location of the centriole (centrioles) varies somewhat according to the cell. It lies generally beneath the cell surface. The pericentriolar bodies are not always present ; they may be associated with the centriole at one time but not at another. Whatever their locations, centrioles are usually seen as paired cylinders, 3000 to 5000 A° long and 12000 to 15000 A° in diameter, opens at one or both the ends and lying at right angles to each other. <u>It is supposed that the centrioles and basal bodies are the</u> [illegible] and their identity lies in the submicroscopic [illegible] has longi- [illegible] les [illegible] When associated, [illegible]

outer fibrils may be triplet as in basal bodies of protozoans. In these cells, the connections between centriolar fibril and ciliary or flagellar fibrils have also been noted. Such connections are quite evident between the animal sperm flagellum and the centriole from which it arises. The centrioles are also found in cells devoid of cilia or flagella.

ORIGIN

There are good number of evidences, which suggest that cilia and flagella are derived from basal bodies and that the basal bodies have the capacity to reproduce themselves. In most ciliates, the number of the basal bodies in a row increases during the growth of the individual from one cell division to next, indicating some process of replication. The mechanism of this self duplication is not fully known. It is definitely not because of fission.

SUMMARY

Cilia, and flagella are not universal cell organelles but are found in many animal and plant cells. *Cilia* are short motile organelles, generally present in large numbers, covering the surface of ciliated cells. Flagella are similar but larger and less numerous, as well as more complex in their movements. Nine sets of paired peripheral fibres surround two longer centrally placed fibres.. They are, however, all visible in cross-section. The peripheral fibres (nine) are double in their structure and the central are single. The entire axial complex is enclosed in a double membrane envelop which is continuous with the plasma membrane.

The cilium or flagellum may be connected with cytoplasmic particles embedded in the clear layer of cytoplasm just between the plasma membrane. To this cytoplasmic particle several names have been given in a variety of plant and animal cells, such as kinetosomes, blepharoplast, basal granule and basal body. The most widely accepted term at present is the basal body. These are however, arranged uniformly in parallel rows under the cell surface.

The location of the centriole varies somewhat according to the cell. It generally lies below the cell surface. The pericentriole bodies are not present, however, they may be associated with the centriole at one time but not at another. The centriole are also found in cells devoid of cilia or flagella.

12

NUCLEUS

Brown (1831) suggested for the first time that nucleus is a constituent part of the cell. Since then, a considerable progress has been made in karyology (—the branch of Cytology which deals with nucleus). It is said to be the major seat of heredity and of the control of all cellular activities. It is a directing and organising unit without which the cell could not exist. Belar has defined nucleus, as "any formation surrounded by cytoplasm from which chromosomes arise during division."

Every cell passes during life through two phases, *i. e. an inter-*hasic or metabolic one and a mitotic one or period of division. Both hases are characterised mainly by the changes in the nuclear *struc-*ure. In the former the nucleus is in the resting stage, *i.e.* in its usual tate of "nondivision" whereas in the latter all changes leading to he division of chromosomes and the reconstitution of daughter *nuclei* ake place. In this chapter we shall confine ourselves to *the* tructure of interphasic nucleus.

MORPHOLOGY

A typical cell of higher animal or plant contains a single ucleus. There are cases where more than one nucleus are found in cell. In plants such cells are called the coenocyte (*e. g.* certain gae like *Vaucheria* and fungi) whereas in *animals* (*e. g.* striated uscle fibres) the term syncytia has been given to such cells. Some otozoans, like *Plasmodium* pass through *syncytial* stages in eir development. In lower organisms, the distinct *nucleus* may, wever, be absent. In some flagellates and infusorians the *nucleus* represented by granules of chromatin, scattered throughout the toplasm. Further in bacterium no distinct *nuclear structure* is servable by ordinary methods. Only recently the nucleoid bodies th the characteristic nuclei have been reported in bacteria.

Position—The position of nucleus in a cell is *variable and in* neral, characteristic for each type of cell. In embryonic cells, it vays lies in geometric centre of the cell which is later on displaced differentiation advances. Thus in adipose cells or in eggs rich in

yolk, it is forced to lie on the periphery. In glandular cells, it lies in the basal region.

Shape—The shape also varies according to the cell type. It is generally spherical but it may be completely irregular in some cases as in leucocytes. In cylindrical, prismatic cells it is elliptical ; in squamous cells it is flattened. Other variations in shapes like horse-shoe shaped, branched, lanceolate, pyriform, etc. have also been observed in other cells. Its shape is not necessarily be related to the shape of the cell.

Size—The nucleus is also not of the same size, but in general a ratio exists between the nuclear volume and the volume of the cytoplasm and this is characteristic for each cell type. This can be [illegible]

[illegible] division.

$$NP = \frac{V_n}{Vc - V_n}$$

V_n = Nuclear volume.

Vc = Volume of the cytoplasm.

Nuclear size is a function of chromosome number. A cell with two sets of chromosomes in the nucleus is called the diploid but when one set is present the term haploid is used. The haploid number is constant for each species and vary from 1 to 800. Since the number of chromosomes has a direct bearing on the amount of DNA in the nucleus, the size of the nucleus is also correlated with the DNA contents.

STRUCTURE

Whatever the forms and shapes of the nuclei, all are more or less complex in organisation and as in cytoplasm, there are well defined categories or components found in them. The notable components are as follows :

1. Nuclear membrane or envelop or Karyotheca.
2. Nuclear sap of karyolymph.
3. Chromatin.
4. Sex chromatin.
5. Nucleoli.

1. Nuclear membrane—It is a delicate but well defined structure that divides the cell into its two main parts, *i. e.* the nucleus and the cytoplasm, both having different physical and chemical structure,

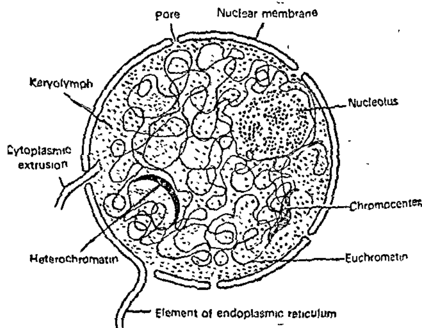


Fig. 60. Structure of the nucleus.

During the cell division, it breaks the cytoplasm become very intima it is reconstituted.

Under the electron microscope it appears to be formed of two membranes, each being 90 A° thick and the shape between the two, the perinuclear s brane structure i ings, each pore pores the two membranes are continuous. These pores are covered by a thin membrane and encircled by a cylindrical wall, which has been called annulus by several authors. Watson (1959) called them pore complex. It has been suggested that these annuli are the cytoplasmic material extending through the pores, not the specialized organisations of the nuclear membrane around the pores.

At many places the nuclear membrane joins the membranes of the tw ndicate the probable origin of nuclear membrane from the endoplasmic

reticulum. But, however, this is a debatable issue. Norman Cohn (1964)suggested that for convenience it is better to regard it as a part of the nucleus. There are, however, some apothical cells of Ascomycete fungus, *Mollisia* where the nuclar membrane has been found directly connected with plasma membrane, suggesting the former's origin from the letter at least in such primitive cells. Chemically also the nuclear membrane is composed of protein and lipid substances like that of plasma membrane.

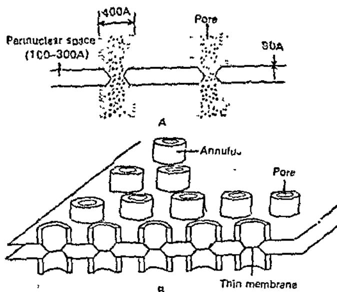


Fig. 61. Structure of the nuclear membrane. A—double membrane and pores. B—pore complex or annuli.

1. Nuclear sap—Inside the nuclear membrane, is found the nuclear sap or karyolymph. Into it are found scattered other nuclear constituents. It is somewhat a granular and homogeneous fluid which escapes if nucleus is broken or punctured. It is composed of primarily protein materials and is probably the site of certain enzymes in the nucleus. It is also supposed to contribute to the formation of spindle during cell division in plants, but now this view is discarded. Recent studies suggest that the spindle meterial is derived from the cytoplasm.

3. Chromatin—The chromosomes as such are not visible in [illegible] ...mes exhibit little [illegible] entangled them- [illegible] Because of the tangling of the strands, and owing to the hydration, they make the

nucleus granular. The cl [illegible]
particularly basic Fuchsir [illegible]
for DNA. According to [illegible]
the Feulgen positive meterial observed in the interphase nucleus and later during the division of the nucleus and is thus a general term for the substance of the chromosomes."

It has been observed that cer[illegible]
darker than the others. These regic[illegible]
regions or hetrochromatin. In th[illegible]
observable which are termed, the chromomeres. Some authorities believe that heterochromatic regions have higher RNA contents than the euchromatic regions or non-heterochromatic regions. In some cases large areas of the nucleus were found to take very dark stains with basic fuchsin. This area is [illegible]
guished from chromomere [illegible]
centre represents heterochı [illegible]
in some cases of all the chromosomes in the nucleus. The gland cells of *Drosophila, Sciara* represent a very good example of this. The study of heterochromatin in these organisms has revealed differences in the rate of RNA synthesis between the euchromatic regions and the heterochromatic regions of the chromosomes.

4. Sex chromatin—Sex chromatin bodies or Barr bodies are the specific heterochromatic bodies, found in many types of mammalian cells at interphase. Their presence helps in determining the sex of the individual. It is most often found near the periphery of the nucleus or near the nuclear membrane. But they may also be present at other locations, depending upon the species or cell type. *It is possible that the different types of cell in the same animal may have different locations of sex chromatin body or bodies.* The average diameter of the body vary from $0{\cdot}8\mu$ to $1{\cdot}1\mu$.

There is usually one sex chromatin body in the nucleus for one diploid sets of chromosomes. The number of chromatin body *will increase with the increase of the number of chromosome sets.* For example, in human female the nucleus containing four sets of chromosomes (tetraploid) possess two sex chromatin bodies. Thus we can conclude that with the doubling of chromosomes in the nucleus, there is a doubling in the amount of sex chromatin.

5. Nucleolus—The nucleolus is a relatively large, generally spherical ball-like body found inside she nucleus. It is generally been considered as having an homogeneous structure, in spite of

the fact that some authors (Simarro, Cajal) have described the presence of small corpuscles with in it. The number of nucleoli vary in different cells and depend on either the species or the sets of chromosomes present in the nucleus. In many plant and animal cells, there is one nucleolus for each haploid set of chromosomes. But in others their number may be two or more for each haploid set. For example in man there are two pairs of nucleoli in each diploid nucleus. Hence the number of nucleoli may be correlated with the number of sets of chromosomes. Their location within the nucleus depends upon the chromosomes or the chromosome area associated with it.

The nucleolus is formed of two different parts, *i. e.* an amorphous part, the pars amorpha and a filamentous part, the nucleolonem. T [illegible] ivision whereas t [illegible] as a part of ch [illegible] there are small bodies having the same structure. These can be said as nucleolar material derived by fragmentation of the outer portion of the nucleolus. The nucleoli consists largely the RNA. Chromatin particles are often attached to them. These may be very large in certain types of cells, such as neurones, the cells which form blood cells, and most growing cells.

During mitosis nucleoli undergo cyclic changes. The nucleoli of the interphase nucleus seem to disappear at the beginning of cell division, *i. e.* prophase at the same time as the chromosomes increase their staining property. At the end of the division, *i. e.* telophase the nucleoli reappears. Each nucleolus lies in contact with a [illegible] the point of union a special region to [illegible] nizing region has been given. The [illegible] derived in telophase from all the chromosomes present, but it is accumulated and organized only in the region of nucleolar-organizer.

The nucle [illegible] thesis. This re [illegible] This synthesis [illegible] is supposed to be controlled by chromosomal DNA. It is further believed that it occurs in the vicinity of chromosomes and then transferred into the nucleolus. The transfer of RNA in the nucleolus can be experimentally shown by the radio-active isotope.

Physiochemical properties of Nucleus

1. In general, the specific gravity of the nucleus is greater than the basic cytoplasm. In some echinoderm eggs, however, the nucleus is lighter than the cytoplasm. Out of all the nuclear components, the nucleolus is said to have the greatest specific gravity.

2. The viscosity of the nucleus is variable.

3. The nuclear pH is more alkaline than that of cytoplasm with practically very little buffering power.

4. The nucleus does not possess the repairing power as found in cytoplasm. The latter, if ruptured superficially, a new membrane is formed in the presence of Ca^{++}ions. If the nucleus is ruptured, the nuclear sap flows out and the nucleus collapses without showing any sign of repairs. This difference in behaviour might be due to the differences in electric charge. The cell membrane generally has negative charge whereas the nuclear membrane has a possitive charge. For this reason, the latter could not combine with cations such as the Ca^{++}ions.

CHEMISTRY

The chemical analysis of the nucleus has been made with the help of *spectrophotometric methods*. This suggests that nucleus has a complex chemical organisation in which the nucleoproteins are the most important components, comprising nucleic acid and specific types of proteins. Besides, other things are also found. To summarize, the chemical composition of nucleus includes :

1. Basic proteins, *i. e.* protamines and histones.
2. Non-histone acid proteins of large molecule weight, *i. e.* residual proteins, chromosomes and enzymes.
3. Nucleic acid comprising desoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
4. Lipids.
5. Inorganic enzymes.
6. Nuclear enzymes.

1. **Protamines and Histones**—Protamines and *histones* are simple basic proteins, having low molecular weights. Protamines may have a molecular weight as low as 2000, and the predominant basic amino acid is arginine. Histones contain three *basic amino-acids*, particularly lysine and arginine Histones are widely *distributed* among cells whereas protamines are more *restricted for* example to the sperms of certain animals.

2. **Nonhistone Protiens**—This protein contains tryptophan

and tyrosine and possess acidic properties which are usually equated with the residual proteins of the chromosomes, which contain more RNA than DNA. The residual proteins differ from histone by its higher tryptophan contents and found in variable amounts in different cells, depending on their physiologic conditions.

It has been suggested that nucleohistone is concerned with the maintenance and reproduction of the chromosomes where as nonhistone components are involved in special metabolic functions of the nucleus.

3. **Nucleic acid**—The nucleic acids, *i. e.* DNA and RNA form most of the nucleus. RNA constitute only 1 to 2% of the dry weight, occuring mainly in nucleolus and in small portions of the chromosomes. The DNA contents of nucleus is indirectly related to the chromosome number. DNA contents per chromosome is *constant and this doubles during interphase prior to mitosis or* meiosis. Nucleic acids are thought to be essential in protein synthesis.

4. **Lipids**—This comprises 3 to 10% of the nuclear mass and occur mainly as **lipoprotein** or **phospholipids**. The phospholipid of the nucleolus differs from that of chromosomes.

5. **Inorganic and other compounds**—Though found in less amount, they are of great biological sifinificance. It has been suggested that the nucleus has a greater concentration of ash than the cytoplasm. The ash is mainly composed of phosphorous, potassium, sodium and particularly calcium and magnesium. In chromatin, these are found in greater proportion. Calcium is the major mineral constituent of nucleus and probably found combined with DNA.

6. **Nuclear enzymes**—These are the most interesting components of the nucleus. They fall into two classes. Some have general distribution and others are found in certain tissues. In the first group, only the enzymes related to nucleoside metabolism (adenosine, diaminase, nucleoside phosphorylase, guanase, etc.) are included. Out of these nucleoside phosphorylase is particularly important as it is found only in the nucleus. This enzyme is involved in the synthesis of coenzyme DPN. Other enzymes, like esterases are found in varying concentrations ; alkaline phosphatase, nucleotide phosphatases are either absent or present in low concentrations. Special enzymes, catalase and arginase seem to be concentrated in some nuclei but are lacking in others.

It is interesting to note that no essential respiratory enzyme such as cytochrome oxidase and succino-dehydrogenase are found in the nucleus ; on the other hand some glycolytic enzymes such as *aldolase*, enolase, are *found. This led to the conclusion* as suggested by Stern (1955) that the cell nucleus may have a predominent anaerobic metabolism that uses glycolysis as its main source of energy.

FUNCTIONS

The functional significance of the interphasic nucleus can be understood by studying the nucleus-cytoplasm relationship. In the interphase, the nucleus and cytoplasm are separated by a membrane which has the *characteristics of permeability. In this condition* the nucleus is incapable of an autonomous existence—a fact which suggests that their inter-relationship is necessary for the maintenance of [illegible] can carry out cel [illegible] (A [illegible] etc . [illegible] inc [illegible] are mainly localized in the cytoplasm and are practically absent in nucleus, it seems obvious that the nucleus depends on cytoplasm at least for its energy requirements.

Several experiments tend to show that interphasic nucleus has a continuous action on the cytoplasm by providing a product which is constantly transported away from the nucleus. The nature of this product is unknown, but whatever be its molecular size, it can easily pass through the nuclear membrane.

Brachet (1957) suggested that the synthesis of RNA of the cytoplasm is under nuclear control. Maiza and Hirshfield (1950) showed that nucleus plays an important part in the uptake and turn over of this element by the organic constituents of the cytoplasm. All these facts lead us to conclusion that continuous interchanges of *substances* exist *between the nucleus and cytoplasm* and that these permit the maintenance of the equilibrium of cellular function and the normal synthesis of the protoplasm.

SUMMARY

Most cells contain a small spherical body, the nucleus whose shape, size and position vary in cells. It remain enveloped by its own membrane, the nuclear membrane. It is formed by two membranes, each being 90Å°

thick. The nuclear membrane is porous, each pore being 400A° in diameter. It provides a pathway for the transport of materials between the nucleus and the cytoplasm. Karyolymph is some what granular and homogeneous fluid.

The chromosomes are the vital components of the nucleus, which at rest are not observable as such but occur in the form of chromatin material. During cell division it organizes itself as individual chromosomes. In chromatin, chromomeres and chromocenters are observable.

Chromosomes are DNA. RNA formed under the control of DNA moves out of the nucleus into the cytoplasm where it acts as a template for the syathesis of protein. RNA is concentrated in nucleous,a large rounded body found in the nucleus. In some cells sex-chromatin are also found. These are specific chromatic bodies.

The nucleus is a major seat of heredity and of the control over all synthetic reactions in the cell. If it is removed, the cell dies. This clearly indicates that the cell cytoplasm is dependent upon or regulated by the nucleus.

It is interesting to note that no essential respiratory enzyme such as cytochrome oxidase and succino-dehydrogenase are found in the nucleus ; on the other hand some glycolytic enzymes such as aldolase, enolase, are found. This led to the conclusion as suggested by Stern (1955) that the cell nucleus may have a predominent anaerobic metabolism that uses glycolysis as its main source of energy.

FUNCTIONS

The functional significance of the interphasic nucleus can be understood by studying the nucleus-cytoplasm relationship. In the interphase, the nucleus and cytoplasm are separated by a membrane which has the characteristics of permeability. In this condition the nucleus is incapable of an autonomous existence—a fact which suggests that their inter-relationship is necessary for the maintenance of a norma' ... gh can carry out cell activit ... l ingesting food (*Amoeba*), ... ia (ciliated cells), etc., but in general they survive only for a short duration and are incapable of growth and reproduction. Since the oxidative enzymes are mainly localized in the cytoplasm and are practically absent in nucleus, it seems obvious that the nucleus depends on cytoplasm at least for its energy requirements.

Several experiments tend to show that interphasic nucleus has a continuous action on the cytoplasm by providing a product which is constantly transported away from the nucleus. The nature of this product is unknown, but whatever be its molecular size, it can easily pass through the nuclear membrane.

Brachet (1957) suggested that the synthesis of RNA of the cytoplasm is under nuclear control. Maiza and Hirshfield (1950) showed that nucleus plays an important part in the uptake and turn over of this element by the organic constituents of the cytoplasm. All these facts lead us to conclusion that continuous interchanges of substances exist between the nucleus and cytoplasm and that these permit the maintenance of the equilibrium of cellular function and the normal synthesis of the protoplasm.

SUMMARY

Most cells contain a small spherical body, the nucleus whose shape, size and position vary in cells. It remain enveloped by its own membrane, the nuclear membrane. It is formed by two membranes, each being 90A°

thick. The nuclear membrane is porous, each pore being 400A° in diameter. It provides a pathway for the transport of materials between the nucleus and the cytoplasm. Karyolymph is some what granular and homogeneous fluid.

The chromosomes are the vital components of the nucleus, which at rest are not observable as such but occur in the form of chromatin material. During cell division it organizes itself as individual chromosomes. In chromatin, chromomeres and chromocenters are observable.

Chromosomes are DNA. RNA formed under the control of DNA moves out of the nucleus into the cytoplasm where it acts as a template for the syathesis of protein. RNA is concentrated in nucleous,a large rounded body found in the nucleus. In some cells sex-chromatin are also found. These are specific chromatic bodies.

The nucleus is a major seat of heredity and of the control over all synthetic reactions in the cell. If it is removed, the cell dies. This clearly indicates that the cell cytoplasm is dependent upon or regulated by the nucleus.

13

CHROMOSOMES

Chromosomes (GK. *Chroma*, colour, *soma*, body) are the most significant component of the cell, particularly during mitosis and meiosis. Their presence was demonstrated long before they were named "chromosomes" by Waldeyer in 1888. They carry the genes and play a major role in heredity. When reproduction occurs, they are passed on to the next generation through the gametes. Besides, they play an important role in variation, mutation, and evolution and in their control of morphogenesis, multiplication and the equilibrium of vital processes. Their physio-chemical composition is such that they have specific and profound effect upon the course of development and hence upon the organism's characters. A chromosome can be considered as a nuclear component having special organisation, individuality and function. It is capable of self reproduction and of maintaining its morphologic and physiologic properties through successive cell divisions.

MORPHOLOGY

The morphology of a chromosome can be best studied at the metaphase or anaphase of mitosis when they are present as definite organelles, being most condensed or coiled.

Size—The size of chromosomes is generally constant. The relative number of chromosomes generally differ in the nucleus but at a time all chromosomes of a cell may be of the same size. They generally appear as cylindrical bodies which vary from $0{\cdot}1\mu$ to about 30μ in length and from $0{\cdot}2\mu$ to 2μ in diameter. Plant cells normally possess larger chromosomes than animal cells. *Trillium* has chromosomes which may reach upto the length of 32μ at metaphase.

Shape—According to the position of centromere the chromosomes are classified into three types : (i) acrocentric—rod-like chromosome having a very small arm and other very long. The centromere mainly terminal ; (ii) submetacentric—chromosomes having unequal arms resembling ∠ in shape ; and (iii) metacentric—chromosomes having equal or almost equal arms and thus are V-shaped. Different chromosomal types are constant for each

specific chromosomes and may also be constant throughout a species or even a genus. Thus the shape of chromosomes is of extreme value and importance for their individualization.

Number—The number of chromosomes serves as an aid in the determination of phylogenetic status, such as taxonomic position of plant and animal species. Their number is generally constant in a species; all members of

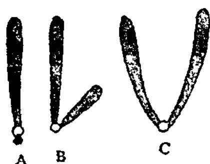


Fig. 62. Different types of chromosomes according to the position of centromere. A—acrocentric ; B—submetacentric ; C—metacentric

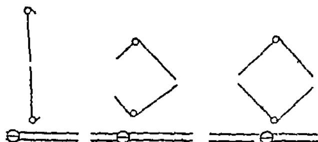


Fig. 63. Diagrammatic representation of V-J-and rod-shaped chromosomes.

that species possess the same diploid number in their body cells and the same haploid number in their gametes. Each haploid set of chromosomes is designated by n and diploid by 2n.

In most of the organisms that reproduce sexually, the chromosomes regularly occur in pairs, of which one member has come from the female and the other from the male. The number of pairs varies in somatic cells from 2 *(Ascaris megalocephala)* or 3 (some green plants like *Crepis* and *Drosophyllum* and some species of *Drosophila*) upto 100 in the crayfish and even more in some ferns and protozoans (approximately 1600 are found in *Aulacantha*, a protozoan). In man the normal number was previously supposed to be 24, but critical studies suggest their member to be only 23.

In some organisms, particular types of cells have usually large number of chromosomes as a result of duplication of the chromosomes without cell division. The chromosomes divide but they do not separate into daughter nuclei but accumulate with in a single nucleus. This process is called the endomitosis. The haploid set

of chromosomes inherited is generally called genome. Thus there are two genomes in a diploid cell, the chromosome of the one genome has a partner or homologue in the other genome. Homologous chromosomes are identical in size and carry similar identical genes. The whole collection of chromosomes in a nucleus is refered as chromosome compliment.

TABLE 4—THE HAPLOID NUMBER OF CHROMOSOMES IN SOME ANIMAL AND PLANT SPECIES

Animal	Haploid chromosome number
Ascaris megalocephala (round worm)	1
Ascaris lumbricoides (,,)	24 (in male)
Bombyx mori (silk worm)	28
Canis familiaris (dog)	39
Drosophila melanogaster (fruit fly)	4
Gallus gallus domesticus (chicken)	39 (in male)
Homo sapiens (man)	23
Ratus norvegicus (rat	21
Pisum sativum (garden pea)	7
Helianthus annuus (sun flower)	17
Allium cepa (garden onion)	8
Solanum lycopersicum (potato)	24

Stebbine suggested chromosome set or karyotypes to be either symmetrical or asymmetrical. In the former, all the chromosomes are approximately of the same size and are all equal armed metacentrics. The asymmetrical karyotypes include elements of different sizes or in which some of the chromosomes are J-shaped or acrocentric. In the animal kingdom, it is diffiult to apply this concept as here the asymmetrical karyotypes are almost universal. But in plants, however, particularly in higher plants ; Stebbins suggested symmetrical one as primitive and the asymmetrical ones as derivatives.

STRUCTURE

During metaphase, when the chromosomes are well defined, each appears t[illegible] matrix and two chromatids. T[illegible] limited from the outside by [illegible] e matrix and

sheath are composed of nongenestic material. The structure and functions of matrix is not fully known.

The two **chromatids** or genonema are held together at a point along their length which is called the **centromere or kinetochore** or **primary constriction.** Each chromatid is formed of two thin longitudinal chromatin [illegible] chromonemata that are supposed to form the gene bearing portion of the chromosome. The portions of the chromosome on either side of the centromere are known as arms. These arms may be equal or un-equal depending on the position of centromere—which is constant for a given chromosome. Chromosomes [illegible] some of a genome has one secondary costriction in one arm. The small segment of the chromosome distal to this constriction is called satellite. Certain secondary constrictions are intimately associated with the formation of the nucleoli though tyey are not usually distinguishable from other secondary constrictions. These specialized regions are termed as nucleolar zone or nucleolar organizer.

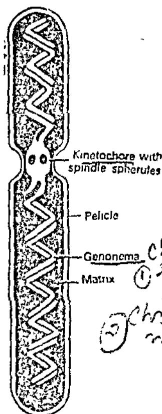


Fig. 64. Structure of chromosome in mitosis.

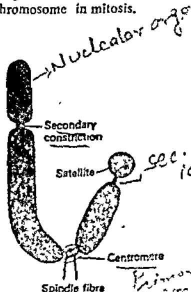


Fig. 65. Metacentric chromosome in external view.

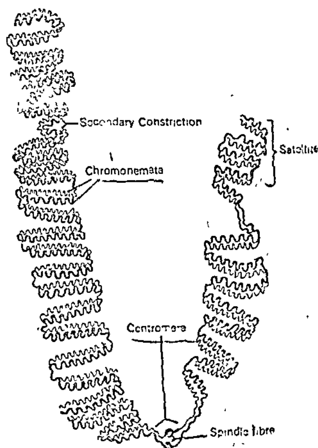


Fig. 66. Metacentric chromosome showing the inner structure with two chromonemata and major and minor coils

Centromere—It is an indispensible portion of the chromosome which is responsible for the shape of chromosome. It determines the shape of the chromosome and is located at the point
where the arms of a chromo ... ted to
chromosomal movement wh ... cture
is difficult to see. It may ... with
large diameter as in maize ... nules
as in *Tradescantia*. The basic structure of centromere includes a clear zone with one or more small granules or spherule (chromomeres). The number of fibrillae in a centromere varies but usually only a few are visible with ordinary microscope. Its morphology

resembles with rest of the chromosome with some notable differences. It is a constriction in a chromosome which stain lighter than [illegible] during metaphase and anaphase. The [illegible] coiled. Darlington (1945) and Schrader [illegible] nilar to centriole with respect to their

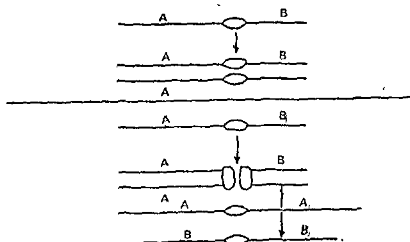


Fig. 67. Diagrams to illustrate misdivision of the centromere and the origin of isochromosome. Above—normal plane of division in the longitudinal axis of the chromosome : Below—transverse division of centromere giving rise to isochromosome A A and B B

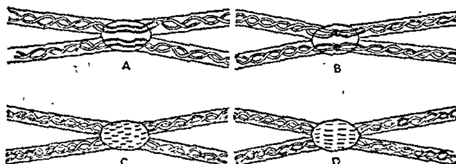


Fig. 68. Hypothetical structure of the centromere in a chromosome with two pairs and chromonemata A—centromere with essential organs of movement ; B—a spindle spherule connected to the chromonemata ; C—a number of oriented micelles ; and D—a number of oriented micelles arranged in such a way as to facilitate misdivisions to a transverse instead of a longitudinal plane.

structure was first observed by Baranetzky (1880) in the pollen mother cells of *Tradescantia*. This filament was named chromonema by Vejdovsky in 1912. The number of filaments involved in the formation of chromonema is not fully known. It may be composed of two, four or more filaments during telophase. The number of

A

B

Fig. 69. Diagrams of Parenemic (A) and Plectonemic (B) types of coils.

fibres may also differ between different tissues in the same organisms. Kaufmann (1948) and Schrader (1948) suggested that chromonema may have single fibre during one stage and two or four stranded during another stage in development. Two or more chromonemal fibres are coiled together; from their coiling two types of spirals origin... nits are freely separable and ... are not easily separable. In ... drawn out, turn into a so called relational coil.

The degree of coiling in meiotic or mitotic chromosome is variable and depends upon the length assumed by the chromosomes during cell division. The meiotic chromosome is composed of two distinct coils, *i. e.* major coil with 10 to 30 gyres, and a minor coil with many more gyres : the latter lie perpendicular to the former. In somatic chromosomes, a helical structure similar to the major coil of meiotic chromosomes has been observed and named standard or somatic coil.

Heterochromatin and Heteropycnosis—In most animals and plants, it has generally been seen during various stages of mitosis that certain chromosomes or segments of chromosomes are more condensed than rest karyotype. This phenomenon is called the

behaviour during mitosis, their appearance in living cells and their staining reactions. In the chromosomes of *Trilium*, the centromere has a diameter of 3μ.

Generally one centromere is located in one chromosome (monocentric) but there can be two (dicentric) or more (polycentric) in a chromosome. White (1936) demonstrated polycentric chromosomes in *Ascaris megalocephala*. In hemipterous insects, the centromere is diffused, found distributed along the entire length of chromosome.

Secondary Constriction—Besides, primary constriction, the chromosome does possess one or more secondary constrictions which differ from the former by the absence of marked angular deviation of the chromosomic segments. These constrictions have been termed **olistherozones** by Resende (1945). They are very useful in the identification of the particular chromosomes in a complex, *i. e*, karyotype.

Telomeres—The chromosomes terminate at either end in a fine structure usually called as telomere, (GK : *telo*, far) a term used by **Mullar** (1938 to indicate the uniqueness of this portion. Telomeres [illegible] been observed that fractu [illegible] but the reuniting pieces [illegible]. It seems as if telomeres have a polarity which prevents other segments from joining with them. In meiotic prophase, the telomeres are often attracted to the centriole and seen to migrate to the nuclear membrane near the centriole. This behaviour results in what is often described as a **bouquet stage**.

Satellites—In certain chromosomes the secondary constriction marks the formation of satellite The satellite is a round elongated body separated from the rest of the chromosome by a delicate chromatic filament Its diameter may be the same as that of chromosome or much smaller. It is customary to designate as **SAT-chromosomes** those which possess satellite. The satellite and the filament are alway constant in form and size for each particular chromosome.

Fine structure of chromosomes—Generallly the chromosomes at metaphase and anaphase do not show any internal structure under the microscope beyond the centromere, constrictions, and satellites. But in the less compact stages or by special treatment, the chromosome was observed to comprise a coiled filament lengthwise. *This*

structure was first observed by Baranetzky (1880) in the pollen mother cells of *Tradescantia.* This filament was named chromonema by Vejdovsky in 1912. The number of filaments involved in the formation of chromonema is not fully known. It may be composed of two, four or more filaments during telophase. The number of

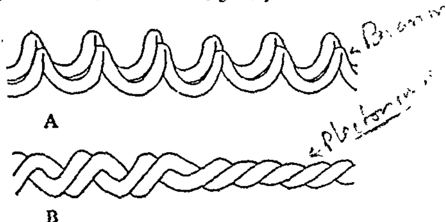


Fig. 69. Diagrams of Parenemic (A) and Plectonemic (B) types of coils.

fibres may also differ between different tissues in the same organisms. Kaufmann (1948) and Schrader (1948) suggested that chromonema may have single fibre during one stage and two or four stranded during another stage in development. Two or more chromonemal fibres are coiled together; from their coiling two types of spirals origin[illegible] coil where the subunits are freely separable and [illegible] are not easily separable. In [illegible] drawn out, turn into a so called **relational coil.**

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Heterochromatin and Heteropycnosis—In most animals and plants, it has generally been seen during various stages of mitosis that certain chromosomes or segments of chromosomes are more condensed than rest karyotype. This phenomenon is called the

heteropycnosis. This may be positive (over-condensation) or negative (under-condensation). It has also bsen observed that the same chromosome or a portion which exhibits heteropycnosis at one phase of its cycle show no or negative heteropycnosis at another phase. The chromosomal material which shows heteropycnosis is referred as **heterochromatin** and the regions which do not show are called **euchromatin**. Heteropycnosis, though characteristic of sex-chromosomes of many species, yet has also been observed in other chromosomes. It may be localized in certain segments at the ends or may effect almost the whole chromosome.

Chromomeres—On the chromonemata, small bodies with constant sizes and positions have been demonstrated. These bodies are called the **chromomeres**; the regions in between them are called the inter chromomeres. Some consider them to be structurally different from the reminder chrómonema because of their ability to synthesize or accumulate in themselves the great amount of stainable nucleic acid. **Belling** (1928) considers them to be genes or group of genes supported in a nongenetic fibril. **Ris** (1957) suggested chromonema as microscopically uniform thread with chromomeres being constant expressions of the coiling impressions. Chromomeres alternate with interchromomere regions, and there is very interesting feature, *i. e.* a portion which is chromomere at one phase changes to interchromomere at the other. White (1936) suggested that the chromomere and interchromomere regions are the two ends of the same continuous chain and where one finishes and other starts is not distinguishable. This view is further supported by the fact that the chromonema is composed of only DNA at all places.

SPECIAL TYPES OF CHROMOSOMES

1 **Polytene Chromosomes**—In the cells of salivary glands of [illegible] .881) for the first time [illegible] strands. These were named **polytene chromosomes** by **Koller** as they appeared to be formed of many chromonemata. Later, such chromosomes are also observed in other organs and tissues such as foregut, midgut, malpighian tubules of most dipterans. The number of chromonemata in a single polytene varies from 512 to several thousands. The characteristic feature of its large size is that the paired homologous chromosomes show the tendency of synapsis or duplication without cell division.

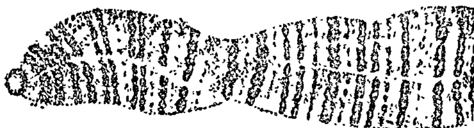


Fig. 70. Fourth polytene chromosomes of *D. melanogaster*.

When stained with chromosomal stain, the giant chromosome shows the characteristic staining pattern. Some regions appear as dark bands of varying widths while other regions, the interbands are often light. The former stain intensely and are Feulgen-positive where as the latter do not stain with basic stains and are so Feulgen-negative and absorbs very little ultra violet light. The interbands also show great elasticity than the bands. The bands are composed of chromomeres of individual chromonemata in a linear array, perpendicular to the axis of the chromosome. The bands are rich in DNA and contain RNA and basic protein as well. The interbands contains less DNA together with acid protein. This banding do not represent a gene but it has been confirmed that the site of pattern has been of particular value in genetic studies. The bands genetic activity in a chromosome of *Drosophila* species lies in the band pattern The interbands have not been studied so intensely, but there are evidences to suggest that they too are genetically active. Breuer and Pavan (1955), and Breemann (1954) suggested that at certain stages of the larval development some specific bands of polytene shows enlargements in the form of 'bulbs', 'puffs', or 'Balbiani rings'. The chromonemata of a band extend laterally to form a series of lateral loops. These loops stretch the chromosome to a wide diameter and thus Balbiani rings appear which give the chromosomes a fuzzy look.

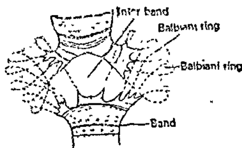


Fig. 72. Balbiani ring in a polytene chromosome.

In short the polytene chromosome of many dipteran flies are well suited for the studies of nucleic acid, gene action and the

correlation of cytological changes with genetic and biochemical changes.

2. **Lampbrush chromosomes**—They were observed in the oocyte nuclei of some of the sharks, amphibians, reptiles and birds quite early in the history of cytology, but quite recently they attracted the

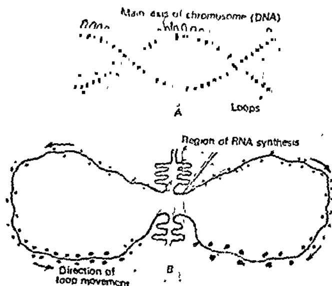


Fig. 72. Diagram of the lampbrush chromosome under low magnification (A) and under higher magnification (B) showing the lateral expansion and spiralization of chromonemata.

attention of scientists. They are quite large and can be detected by naked eyes. Like ploytene they are also giant because of the size of chromonemata. This occurs while the oocyte is maturing. The lampbrush chromosomes is characterised by fine lateral loops extending from their main axes except in the centromere regions. This gives them brush-like appearance (hence called lampbrush chromosome).

Structurally, the central axis of chromosome is probably composed of at least four chromatids to which the lateral loops are attached. The loops represent the lateral extended portion of the chromatids and are covered with matrix which give them fuzzy appearance. The matrix is composed of RNA and protein. Recently Ris (1957) has shown that the loops are bundles of submicroscopic fibrils. At the bases of loops lie the chromomeres which stain dark

and represent the tightly coiled sections of the chromosome axis. Loop axis has been suggested to be 30 to 50 A° in diameter. Gall (1956) described the presence of certain number of DNA strands in each lampbrush chromosome. The function of lampbrush chromosome involves the synthesis of RNA and protein by their loops and probably the formation of certain amount of yolk material for the egg.

3. **Supernumerary chromosomes**—Some plant and animal nuclei, in addition to the normal chromosomes possess one or more accessory or supernumerary chromosomes. They are usually of smaller size than the other types of the chromosomes. They usually produce little detectable phenotypic expression in the organism, in which they are found. Supernumerary chromosomes are relatively unstable member of the chromosome compliment. They can be distinct from other simple types of chromosomes as they do not show the activity of somatic nondisjunction and illumination and are randomly distributed.

CHEMISTRY OF CHROMOSOME

Nucleic acid and proteins are the most important and major chemical constituent of the chromosomes. Quantitatively, the chromosome normally contains about 90% deoxyribonucleo protein, and 10% residual chromosome. The former comprises 45% DNA [illegible] chromosome comprise 12 [illegible] 6% residual protein. The residual protein is an acidic protein and mainly contains large

[illegible]

are removed at least the structure will not be completely demolished. Protein in the chromosome probably serves as a frame work to which the different nucleic acids are attached. It is not possible to separate any of the chromosome constituents, without injuring chromosome structure. So all the components are very important for the full maintenance of structural and functional characteristic in the integrated system of chromosome. In addition to the histone and protamine, there is another type of protein called chromosomin by Stedman and Stedman (1943) which contains high tryptophan contents.

The linkage between the protein and DNA are primarily of

an ionic nature and are usually called salt linkage. Recent investigations, indicate the presence of specific metalic ion in the cells which suggest an additional linkage in the chromosomes. Such divalent ions may be Ca^{++} Mg^{++} and Fe^{++} which are usually found in the nucleus. The research on chromosome breakage and selective incorporation of these ions into the chromosome has suggested that these divalent metalic ions are too essential to the structure of chromosome. These metalic ions may occur in between the DNA and protein or in between the DNA groups. In the later case, they provide the linkage through the phosphate end group of the DNA molecules. Others linkages, however also exist. They involve strong attraction between the negatively charged phosphate groups and positively charged histone protein.

The amount of DNA in the diploid cells is constant for the given organism. However, its quantity differ in different organisms. The DNA content in the liver cells of frog is about seven times greater then the DNA content in the liver cells of chicken. The DNA quantity in the cell is directly proportional to the number of the chromosomes in the nucleus. It can be pointed out here that gametes of an organism have exactly half the DNA content of its somatic cell. In the same way the triploid nucleus contains three times as much DNA as a haploid nucleus. There are so many deviations in the quantity of DNA in the chromosome but these changes can be accounted for changes in the cell metabolism at the time of DNA determination.

DNA is a prim [illegible] several evidences. Firstly, [illegible] ibers are related with eac [illegible] the nucleus and transn [illegible] sion. Finally, the wave-lengths of ultraviolet light that induce mutation are of the same wave-lengths that are absorbed by DNA. *The most* remarkable feature of DNA is that, it has the same *physical and* chemical organisation in all organisms, but provides far *greater* diversity among different animals. The reason of *this diversity* see [illegible] combinations of [illegible] le.

DNA [illegible] cycle—*It is* interesting to observe the changes in DNA during the *life cycle of* the cell. It will determine the exact timing of the reduplication of the DNA during mitosis. In the figure 73 the relationship between

Fig. 73. Diagram to show the changes in DNA during the cellular cycle.

DNA synthesis and the various stages of the life cycle of the cells are illustrated. The diploid content *of DNA is expressed by 2C*. After mitosis the cell may undergo two cycles, *i. e.* (1) it may go into an autosynthetic interphase with
new division, and (2) *it may*

during which there is no duplication of DNA and no cell division. The diagram also depicts the cases of polyploidy and polyteny by endomitosis.

Previously it was believed that DNA synthesis occurs during the mitotic prophase but it has now proved to occur in most cases during the autosynthetic interphase, but the specific time, however varies from organism to organism. It has been reported by some authors that duplication takes place even at the telophase of the preceding divisions, others think its occurence during prophase division. In meiosis it usually occurs before prophase. According to Taylor (1960) the original DNA helix of a chromosome serves as a template for the new helix. The DNA network splits up longitudinally in the two halves, each acts as a template for the synthesis of a new genetic material. Thus DNA has a capacity of self reproduction

The duplication of the chromosomes has been studied in conjunction with the synthesis of DNA in both plant and animal tissues. Experimental findings confirm the model of DNA and its replication proposed by Watson and Crick. Its details has been discussed in the separate chapter.

FUNCTIONS OF CHROMOSOMES

1. The foremost function of the chromosome is to control the physiology of an organism with the help of genes which they carry.

2. The regions within the chromosome can change their position. Such changes often have a genetic or phenotypic effect, upon the organism. This effect has been referred as position effect. This position effect can be because of the shifting in the position of heterochromatin with respect to euchromatin.

3. The heterochromatin region of chromosome takes part in the production of nucleolar material.

4. There exists some indirect relation between the chromosome and the synthetic activities of the cell. The nucleolus possesses RNAwhich serves as a means of transmission of genetic information to the cytoplasm. This leads to the formation of a synthetic product like protein.

SUMMARY

Inside the nucleus, the structure of greatest interest from the point of view of genetics is the chromosomes.

They carry genes and are passed on to the next generation through the gametes. They play major role in heredity. Besides, they play an important role in variation, mutations and evolution. In the interphasic nucleus they are represented in the form of chromatin material but during the cell division, particularly during metaphase and anaphase, they are found as definite organelles, being most condensed or coiled.

Their shape, size and number are generally constant for a species. In most of the organism that reproduce sexually, they regularly occur in pairs, one comming from female and other from male. The number of pairs in a somatic cell vary from 2 (*Ascaris megalocephala*) to 1600 (*Aulacantha*, a protozoan). The whole collection of chromosomes in a nucleus is referred as **chromosome complement.** Typically they are rod-like but some appear to be spherical.

Typical chromosome consists of a fluid **matrix** and two **chromatids,** held together at **centromere.** Each chromatid has generally two longitudinal threads the **chromonemata** which are closely associated together. Chromonema is the basic unit of chromosome and bear the genes. On either side of the centromere, the portions of the chromosome are called arms. The arms may be equal (metacentric) or unequal (acentric or sub-centric). Secondary constriction in one or both the arms marks the formation of **satellite** terminally. **Telomeres** are the terminal ends of a chromosome which possess polarity. Chromonemata are coiled together, giving two types of spirals, *i. e.* **paranemic coils**—which are easily separable and **plectonemic coils.** The plectonemic coils are not easily separable and when drawn out, the **relational coil** results. On the chromonema, small bodies with constant sizes and positions are found. They are called **chromomeres** and are separated by **inter-chromere regions.**

Polytene chromosomes are the giant chromosomes, observed in the salivary glands of *Chironomus* larvae.Each such chromosome has about 512 to several thousand chromonemata. Each polytene shows characteristic staining pattern, being having **bands** separated by **inter-**

14

GENE

[illegible]

some. Further the distance between the two genes in a chromosome

[illegible]

lised and can therefore be observed These are known as visible genes for example the centromeres, and the heterochromatin. Swanson (1963) pointed out that the concept of gene can be recognised only when its mutated form can be compared with the normal or wild type gene from which presumably it arose.

Before a cell divides, the proteins of the gene become associated with nucleic acid which gives them power to grow and reproduce. Genes can grow,and reproduce their own kinds and can also mutate. A gene normally reproduces itself identically, but occasionally one of the genes does not reproduce exactly itself. There may be some gain or loss in it, therefore its actions become different. This causes the gene mutation. It is important to note that though the gene mutations are not uncommon, genes are regarded to be resistant to a change.

Definition—A gene has been variously defined. It is the "ultimate unit of recombination". Genes are those parts of the chromosome between (but not within) which crossing over takes place. The chromosome thus has a linear organisation, the part of which concerned with the determination of different features of the organism. Delineation of character, however may depend upon one gene. The gene has also been defined as the "ultimate unit of mutation This

definition assumes the identity between the gene and the smallest segment of the chromosome, capable of a change that is reflected in the form of detectable phenotypic characters. According to some the gene is " a unit of physiological activity" Qualitatively it is very easy to distinguish between a gene that controls one phenotype and another that controls the other and distinguishable phenotype, but the distinction becomes blurred as criteria become more quantitative or as phenotypes overlap. **Pontecorvo (1952)** adds the fourth definition, suggesting that "**it is the ultimate unit of self reproduction**". **Finally Watson and Crick (1953) and Wilkins (1962)** defined the **gene as a molecule or a large chemical radical of carbon, oxygen, and hydrogen, phosphorus and nitrogen attached with a undifferentiated pr** ... one cell **to other** ... **changing** ...

It must be quite obvious the five definitions cited above are in consistant with each other to varying degrees and that each definiton is meaningful only within the limit of techniques used in studying the genes.

Position effect—A s... ...ible for so many characters and a group o... ...ble for a single characte... ...involved but the positior... ...nes the expression of ch... ...**effect.**

Genic balance—An adult individual is actually not the result of independent action of separate pairs of genes added together but the action and reaction of these pairs of genes with one another. This is called **genic balance.**

Shape of gene—The actual shape of the gene is not known Recently, the discovery of electron microphotography has made it possible to speculate about the shape of the gene Stanley (1952) and Slizynski (1944) contributed much about the shape of the gene. Stanley studied the virus and according to him the virus is rod like structure which resembles a gene in its basic fundamental unit. Slizynski studied the salivary gland chromosomes and showed numerous dark long bands, probably genes. Thus on the above observations it is easy to say that "a gene may be a micro-cylindrical **rod-like unit.**" The target theory of x-ray mutation completely supports the above observations. According to this theory, if a

gene is a small dott-like structure, it will be less exposed to any hitting by X-rays, while if any structure is rod-like it is more exposed to hitting and it may be broken down or any change can be made (mutation) even by the slight hitting.

Position of the gene—It is a matter of great despute that actually where the genes lie on the chromosome. Some light has been thrown by Demerec (1939) on this problem. He pointed out that the most important and most conspicuous part of the chromosome is a chromonema, a thread-like structure which runs along the whole length of the chromosome. [illegible] structures and fuctions.

Size of gene—Since no one has seen the genes, as such their dimensions have not yet been measured directly, although various attempts have been made in this direction. However, it appears that their sizes vary considerably. The diameter of one gene (assuming it to be a spherical particle) has been calculated by "target size estimation". It comes to round about 6 millimicrons and its molecular weight to be perhaps around 100,000. These are questionable because they are based on detectable nonlethal mutations induced at a particular law. Such mutations presumably represent only a fraction of the total changes induced by ionization. Muller (1947) on the basis of four genes located in a limited length of salivary gland chromosome, concluded that they had a mean length of 1520A°, while Pontecorvo considers 4500A° to be an approximation of gene size in *Aspergillus nidularis*.

Stability of gene—It has been pointed out that the chromosome is an extremely stable, though dynamic structure appearing in unchanged form in a particular cell, generation after generation. Giles (1940, 41) on the basis of the study on *Tradescantia*, suggested that the gene stability can be measured on a chromosomal basis. The stability of gene can be studied in terms of half life of a gene, *i.e.* the time elapsing for a 50% probability that a particular gene will mutate, or conversely the time in which 50% of the genes would be expected to mutate. Muller (1960) has calculated that in *Drosophila* a gene, on an average, has a half-life of 10^3 to 10^4 years while in man approximately 10^5 years. Auerbach (1951), Dermerec

and Hanson (1951) pointed out that various types of chemicals can also effect genic stability. The effective chemicals are of varying kinds such as phenol, peroxide of various kinds and manganous chloride. Moreover, there is no hint in their structure or mode and degree of reactivity to indicate that why they in particular are mutagenic while others are not. The hope that the specific heritable mutation has not yet been realized.

Th[illegible] at DNA [illegible] out the [illegible] DNA. How [illegible] ept and definition of the gene has held it to be the smallest unit which can be recognised for recombination, mutation and function. Recent observations on the problem, however, indicate that these are necessarily the same units. Furthermore the microbial genetics and studies on r-mutants in bactriophage have made it possible to show that the [illegible] function are different. [illegible] osed to designate these [illegible]

1. Recon—It is a smallest element which is interchangeable through genetic recombination. The detailed and extremely delicate studies of recombination in microbes indicate that a recon consists of not more than two pairs of nucleotide, may be only one.

2. Muton—It is also the smallest part, which, when altered, can give rise to the mutation. As we already know that the mutation is caused in the alteration of a single nucleotide pair.

3. Cistron—It is the functional unit and the gene in real sense what we think generally. The studies indicate that these can be even more than hundred points within a functional unit wherein a mutation can take place and ca[illegible] effect. This observation indicate that a [illegible] of nucleotide pairs in length and [illegible] that even some cistron may be as long as 30,000 nucleotide pairs.

It can be concluded on the basis of these informations collected recently that it is very easy to understand how pseudoalleles are merely recombinations of recon with cistrons and how multiple alleles are easily possible whenever changes occur in different mutons within the same cistron We should not forget.

* For more details see authors "Text Book of Genetics".

that these new discoveries and the new terms used here, will hence forth abandon the use of the term "gene" but rather now we are more able and more clear about the concept and structure of the gene than previously. Our general consideration, when we refer to the functional unit (*cistron*) when we use the term "gene" without qualifications. It should be emphasized that the genetic units proposed by Benzer are completely operational in meaning and origin. They serve the important function of pointing up the complexity of the word "gene" by making a clear distinction between the several operational components of this word, recombination mutation and function.

DNA, the genetic material*—Since gene themselves have not been isolated and analysed, our conclusion are based on the isolation and analyses of the chromosomes because cytological and genic-evidences give [illegible] The chromos[illegible] study reveals [illegible] of substances, *i. e.* proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) ; all occuring as polymers in nature. Further, the evidences drawn from bacterial transformation, bacterial viruses and many plants and animal viruses suggest that genetic information is coded in DNA of the chromosomes and that RNA is involved in the translocation of information into action. So DNA partially or completely represents a gene. Further researches have found that DNA can be isolated from cells of nearly all living organisms. It was found that independent of the source, all DNA have practically the same chemical and physical properties.

We no longer doubt that DNA is a vital component of the chemical basis of heredity. Whether DNA alone contains all the genetic informations or DNA acts along with other material but it is not yet known what protien associates with DNA for the purpose.

Since there is overwhelming evidences to suggest that the genes are formed of DNA material, so first of all we should know something more about the chemical nature of nucleic acid which plays so much important role in the formation of such a [illegible] microstructure of the life of all organisms. It is a well known [illegible] that DNA [illegible]

[illegible]

*Synth[illegible] [illegible] this book.

up of many simpler organic compounds. Out of these organic compound, one is the pentose sugar (sugar with five carbon atoms) which is possibly linked with the inorganic phosphate (that means, the sugar is phospholated). The sugar in DNA is deoxyribose. The second organic substance is a variety of ring compound bases known [illegible] in DNA molecule has [illegible] the object of intensive research.

It is very important here to point out that the genetic diversity must exist. Although the backbone of DNA molecule, *i. e.* sugar phosphate is the same everywhere. It is only the differences between all forms of life on earth which lie in the different sequences in which these bases are arranged. A theory to explain this structure in the way which accounts for all the known properties of DNA has been proposed by Watson and Crick. According to the theory propored by [illegible] double helix. These two [illegible] molecule connected by their phosphate bonds to form a long string. The purine and pyrimidine bases project inward these two string, which hel[illegible] These are only we[illegible]ey are sufficiently numerous to hold the helix in shape. Its detailed structure and chemistry has been discussed in the chapter of Nucleic acids and Nucleic acid synthesis.

Muller's classification of genes or dosage relation of genes— It has been observed that a given character may be affected by several different genes and a gene may affect the reaction going on in the development of an individual. Thus there is no single one to one relation between a gene and a phenotype characters but such a relation only exists between the phenotype and the genotype as a whole. Sometimes this is referred as the **balance theory of genetic action.** This genotype as a whole other than the particular gene with which we are concerned can be referred to as the **genotypic millieu or the genetic background. Ressovsky (1934)** studied the effect of genetic background on the expression, of a certain genes in *D. funebris* and suggested that the background of different local races may alter both the degree of expression, the penetrance and the actual mode of expression, of certain genes.

Further, this variability in the genotypic millieu depends on

the quantity of genes present. To tackle this problem we should know how the expression of gene is altered firstly when more of it is added to one and the same background and secondly when more background is added to the same quantity of genes. Muller (1932) specially studied this problem and has proposed dividing genes to be of five types. This is generally called the Muller's classifications of genes. It should be kept in mind that this classification refers only the relations betweem genes, not of genes themeselves and is not applicable to those organisms where no standard or wild type is available.

1. **Hypomorphs**—The genes which do the same thing as the standard one but less efficiently are called the **hypomorphs.** If their number is increased in a constant genotypic millieu (by adding chromosome fragments containing it and only a few other genes), the gene effect will increase until sufficient of the hypomorph may be present to give the same effect as the standard.

2. **Amorphs**—A gene when has very little the same effect as the standard gene is called the **amorphs.** The limit of variation in this for gene is to have no effect If they are added to genotype millieu, there will be no alteration in the phenotype. They are the contraries of neomorphs.

3. **Hypermorphs**—These genes do the same thing as the standard genes but do it better. It can be said that hypermorphic relations being the converse of the hypomorphic one.Ressovsky's mutation caused by X-rays from the hypomorphs forked to the wild (hypermorph) forked allelomorph demonstrate the actual possibility of occuring such genes. But in organisms like *Drosophila*, where the wild type is taken as standard, hypermorphs occur rarely.

4. **Antimorphs**—These are the genes which have an effect opposite to that of standard ones. An example of the abnormal abdomen in *Drosophila*, can be taken. The Ab gene has an effect more extensive than a deficiency for the wild type allelomorph. One might say that the gene is doing the same thing as that wild type but with negative efficiency.

5. **Morphs**—These are the genes which do something quite different to anything done by the standard gene. In fact, the standard gene behaves towards them like an amorph.

It is quite clear from the foregoing account that there is no definition of gene that satisfies all experimental situations. The gene

itself is capable of evolving new properties. How this can be accomplished was not known until references to a *chemical model* of gene action was known (Schwartz, 1955), but there is no immediate reason for believing that the properties of the gene in term of physiology, mutation, recombination and reproduction need to evolve in the same direction and at the same rate.

SUMMARY

The term "gene" as used in this context has, untill quite recently, been employed to convey the purely abstract concept of a unit of heredity. It, however, represented a quantum of genetic information that in some way controlled the biosynthesis of a single protein or, in more cautious terms, of some "functional unit". Watson and Crick(1953) and Walkin(1962)define the gene as a molecule or a large chemical radical of carbon,oxygen and hydrogen, phosphorous and nitrogen attached with a undifferentiated proteinous thread, the chromonema. Gene shows the position effect, according to which single gene may be responsible for so many characters and a group of closely associated genes may be responsible for a single character, so, it is not the number of the genes involved but the position of genes in the chromosome that determines the expression of the characters

A gene may be a micro-cylindrical rod-like unit. The dimensions of the gene has not yet been determined, but however, it appears that their sizes vary considerably. It's size is about 6 millimicrons and its molecular weight to be perhapes around 100,000.

Seymour Benzer has indicated the subunit of gene after studying the process of mapping a very large number of mutants of bacteriophage T_4. These studies represent the first real attempt to determine the ultimate limits of *recombination*. The subunits are **recon**—smallest element in the one dimensional array, *i. e.* interchangeable by not divisible by recombination. The **muton** is defined as the smallest element of the cistron that, when altered gives rise to a mutant form of the organism. The cistron is the genetic unit of function which again itself contain so many functional subunits.

Further Mullar classified the gene on the bases of the dosage. The gene may be **hypermorph, amorph, hypomorph, antimorph, and morphs.**

15

MITOSIS AND MITOTIC APPARATUS

It is the fundamental goal and duty of every living being to produce their own image so as to ensure the continuation of species from one generation to another. Two processes, however, govern this phenomena ; first the union of cells and second is the division of cell. The division c [illegible]
organism, while the unior [illegible]
produce sexual cells or ga [illegible]
consists of two phases, *i. e.* growth and division. As a result of anabolism in the tissue or cell, it increases in size, but the limit of growth is fixed and this growth limit is determined by two factors, the nuclo-cytoplasmic ratio and the ratio of the cell surface area of the cell volume (relation of volume and the surface of the sphere:—Volume=4/3 $\pi r3$; surface area=$4\pi r2$).With the help of this formula we can know, that a constant ratio is maintained between the size of the nucleus and the volume of the cytoplasm of the cell and this ratio is controlled by the differant physiological activities of the cell. Nageli (1846) [illegible] by the di [illegible] r-
ted th [illegible] he described the direct method of cell dvision. In 1882, Flemming described under the name mitosis, the series of changes that a nucleus ordinarily undergoes in dividing to form the daughter nuclei. Schleicher (1878) described the nuclear division and called it as karyokinesis (Gr. *Karyon*=nucleus=; *kinesis*=movement). The cell division is of three types :—

1. Amitosis or Direct cell division.
2. Mitosis or Indirect cell division.
3. Meiosis or Reduction division.

AMITOSIS

This division of the cell is of very simple type and it occurs in unicellular animals like protozoans and the cells of foetal membrane of some vertebrates. In this type of division the cell divides

and this process starts with the elongation of the nucleus into a dumb-bell shaped, which then constricts in the middle. This is followed by the splitting of cytoplasm and cell membrane. Cytoplasm gather round each daughter nuclei and the cell eventually separates into two daughter cells. This type of cell division is called **amitotic cell division** or **amitosis**.

MITOSIS

The body of the different multicellular organisms is made up [illegible] ells, namely **somatic cells** and **germ** [illegible] he soma (different parts of the body) [illegible] of life except reproduction. The [illegible] ghter cells of equal size [illegible] of chromosomes as the [illegible] atic cell division. It was [illegible] lamming (1878 and 1882) showing longitudinal splitting of chromosomes during nuclear division. More details about it were worked out by Scheneider (1883). The term **mitosis** (GK : *mitos*=thread) refers to thread-like appearance of chromosomes in early cell division. All the somatic cells of the body divide by this process to form the multicellular body.

Thus 'mitosis' is a characteris [illegible] **maintaining the same number of** [illegible] **present in parent cell.** Every cel [illegible] characterised by the presence of two main components, *i. e.* the chromatic and the **achromatic** apparatus. These two constitute the mitotic figure. The chromatic apparatus is formed by the chromosomes and also by the nucleoli as the latter take part in the mitotic cycle. The achromatic apparatus is formed by the centres or poles and the spindle.

As has already been stated that the mitotic cell division comprises the nuclear division (karyokinesis) which is followed by the division of the cytoplasm (cytokinesis). The karyokinesis can be divided into four stages :—

1. Prophase
2. Metaphase
3. Anaphase
4. Telophase

In some cases, prometaphase does exist between the prophase and metaphase.

Interphase :—

The resting stage of the cell is called [illegible] During this period n [illegible] been listed as a stage of [illegible] when the cell prepares itself for the division; and this necesiates its description also. The period varies from organism to organism. It is the longest period in the cell cycle and is often measured a day. All the metabolic activities of the cell occur at this stage except cell division. The cell at this stage is characterised by the following events.

When the cell stained at this stage both nucleus and chromosomes appear as dark staining bodies. The nucleolus being more pronounced. The chromosomes show minimum degree of condensation or coiling and are so entwined that they can not be distinguished individually. Any coiling at this stage is due to the previous mitotic cycle. The coil at this stage is called relic coil. The significant nuclear events related to the mitosis is the duplication of deoxyribonucleic acid (DNA) and consequently of chromosomes. This DNA synthesis usually starts since late telophase and completed in mid interphase. This stage of synthesis is known as S-stage. The S-stage is proceeded by G_1-stage which is subsequently followed by G_2-stage. Earlier it was believed that DNA synthesis occurs during the mitotic prophase. But now it has proved that this occurs during the autosynthetic interphase when the chromosomes are in the state of highest despersion.

1. **Prophase :**

Prophase is the longest stage of mitosis and lasts from one to several hours. In onion root it lasts for 71 minutes, in grass hopper neuroblast for 102 minutes and in the pea endosperm it takes 40 minutes. Prophase is said to be initiated at the movement when the chromosomes become visible. This stage is marked by the following events. The cell tends to become spherical and increases its refractivity

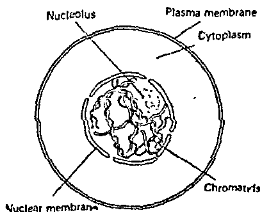


Fig. 74. Mid-prophase.

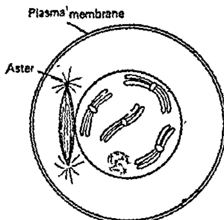


Fig. 75. Late prophase.

and turgidity. This is accom-

that this ball is formed of a single continuous filament, or spireme. This spireme undergoes transverse segmentation, thereby producing a number of pieces or chromosomes of varying lengths. This interpretation has now been totally abandoned, There are several cytologic and genetic proofs suggesting that they (chromosomes) are not the fragments of one filament but each possesses a special differential organization and maintains throughout the entire nuclear cycle its autonomy and its specific function.

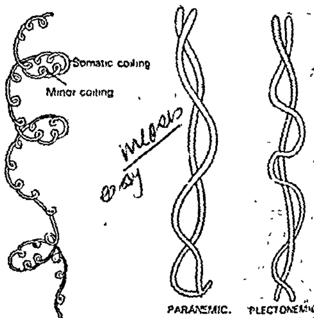


Fig. 76. Diagrams showing the prophase coiling.

Each duplicated chromosome is composed of two **chromatids**, each of which undergoes a regular cycle of coiling. The two chromatids are closely associated along their length but do not actually fuse. They are connected by a single centromere whose position as clear circular zone in a chromosome is constant. Continuous condensation **(dehydration)** results in more and more coiling of the individual chromosome as well as of the two chromatids around each other. The coiling of the two chromatids developed at two levels, the larger ones are **somatic coils** and the smaller ones are **minor coils**. The somatic coils which are smaller at first stage, increase in size, but decrease in number as the prophase progresses. This process happened due to the apparent thickening of the chromosomes. Thus the chromosomes become shortened and increase in thickness. In most of the cases the two chromatids of a single chromosome twisted about each other relationally so much that it is not easy to separate them. This type of association is known as

however, differs from the

be separated laterally.

ing the entire prophase,

l in the nuclear cavity,

This separation increases as the prophase progresses. The chromosomes start approaching the nuclear membrane, leaving empty central space in the nucleus. This movement indicates that the disintegration of nuclear membrane is approaching.

In continuation of these physical changes, some chemical changes also occur in the nucleus. This change mainly involves the increasement in RNA contents of the chromosomes, and increase in their phospholipid contents. According to certain authorities, addition of matrix also takes place in the chromosomes surface. This increases the density of the chromosomes by the accumulation of RNA and phospholipids. In the telophase these substances diminish.

Now the spindle formation takes place. It can be of two types. In one type, the single centriole divides in two daughter centrioles which separate. Each shows an aster or astral rays and between the two asters there appears a bundle of delicate filaments called the spindle. The centrioles continue their migration along with the asters, until they become situated at antipodal positions. In the other type, the centrioles are already polarized before the beginning of the division. The spindle forms at metaphase. The

former is called the central spindle and the latter as metaphasic spindle. The mitosis in which the achromatic figure and spindle are formed by centres, *i. e.* centrioles and asters are called the astral or amphiastral mitosis. This is common in animal cells and some lower plants. Mitosis in which these centres are absent are called the anastral and are characteristic of good number of plants. The nucleolus in most of the cases decreases in size, gets smaller and finally disappears completely.

The final step or event of prophase, is the breakdown of the nuclear membrane. The membrane disappears into the cytoplasm and also in endoplasmic reticulum. However, in most of the higher organism, it is not certain that the nuclear membrane is completely broken down. It may become the part developing spindle and later return to its former condition and position at telophase. A fluid zone at this time can be noted in the centre and the cell in which the chromosomes move freely and in apparent disorder towards the equator.

2 Pro-metaphase :

The disappearance of the nuclear membrane indicates the end of prophase and the start of metaphase. However, there are some protozoans, where the breakdown of the nuclear membrane does not occur upto this stage. Some scientists believe that there exists one more stage between the prophase and the metaphase, known as prometaphase. Swamson (1963) has not mentioned any such term but Cohn (1964) referred this term which includes the movement of chromosomes towards the central region of the cell. White (1963) defined prometaphase, "as the period during which the spindle is being formed, and during which the chromosomes give the impression of struggling and jostling one another in an attempt to reach the equator of the developing spindle." The complete stage of prometaphase and metaphase last to a very short period from 6 minutes to 13 minutes and is characterised by the following events.

As the prometaphase proceeds the chromosomes reach the plane of the equator, where they get arranged radially at the periphery of the spindle ; as if they were repelling each other. In doing so, their centromeres lie on the equatorial plane. This arrangement is characteristic of the animal cells. In plant cells they become irregularly arranged and occupy the entire surface of the equatorial plane of the spindle. This distinction in arrangement is however not always clear.

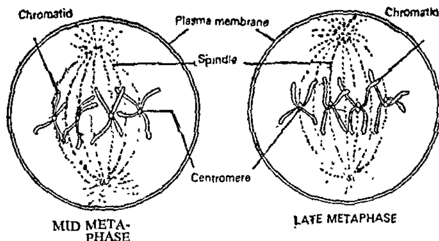


Fig. 77. Stages in metaphase.

3. Metaphase :

When the chromosomes have arranged so, we consider the end of the prometaphase and the start of the relatively static metaphase stage. The metaphase is characterised by the following events.

At the end of prometaphase, the chromosomes have reached to their maximum [illegible] ve acquired smooth [illegible] le, loosely wound [illegible] of separation from [illegible]

The centromere of each chromosome is attached to the spindle fibres. This attachment is instrumental, directing the chromosomes to the cell equator ; their centromere towards the equator and their arms extending freely in surrounding cytoplasm. The fibres of the spindle that connect the chromosomes are called the chromosomic fibres ; those which extend without interruptions from one pole to another are called continuous fibres, The centromeres of the chromosomes divide simultaneously so that each sister chromatid has its own centromere.

Darlington (1937) have separated the whole process into three components, *i*. e. congresson, orientation, and distribution. These processes include the chromosomes from their widely spread condition within the nucleus to a position of equilibrium between the two poles and their later orientation and distribution on [illegible] plate.

4. **Anaphase :**

Metaphase passes into the anaphase at the time when the centromere becomes functionally double, and the chromatids began to move towards the respective poles. It is the shortest of all the stages in the mitotic cycle and is characterized by the following changes.

The daughter centromeres move apart and the chromatids separate, and start moving towards the poles. This movement of chromatids seems to be autonomous. These moving chromatids which have entirely separated from one another are termed as **daughter chromosomes.**

In the late anaphase, the zone between the two groups of chromosomes or equatorial region of the spindle between them elongates and the fibres seem *to be stretched and* are called **interzonal fibres.** The expansion of the middle part has been referred as ***stemmkorper*** or ***pushing*** body by Belar. This pushing body is a kind of gel which pushes the daughter chromosomes towards the poles at the end of the anaphase. However there are other mechanism also for the separation of the daughter chromosomes. There may be a continuous coiling of the chromosomes during anaphase so that they become more and more condensed than at metaphase.

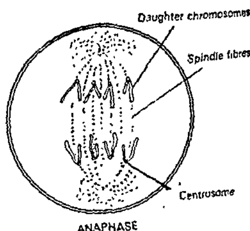


Fig. 78. Anaph ase stage.

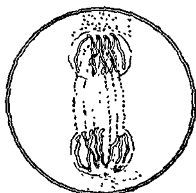
The chromatids assume a definite shape either J or V at the time of movement toward the poles. If they are in the form of J, they are known as hetero-branchial, if of V they are known as isobranchial chromosome.

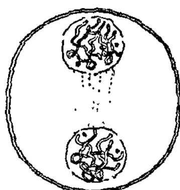
5. **Telophase :**

The end of the polar migration of the two daughter groups of chrumosomes marks the beginning of the telophase. A little later the process of nuclear reconstruction occurs. It is characterised by the following events :

The two groups of chromosomes rapidly loose their smooth

outline and begin to undergo de-condensation. They are usually in a tangled mass at this stage. The nucleus begins to reappear but it is not well known that how it is reformed. The available infor-





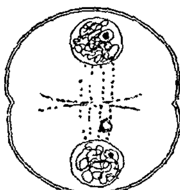


Fig. 79. Successive stages in Telophase.

mation indicate that much of the nuclear material becomes scattered among the chromosomes by the end of prophase and this material is reconstituted during telophase. There are also evidences in which the new nuclear material also develops during telophase as RNA syntheysis. A new nuclear membrane is formed around each group of daughter chromosomes. The new membrane originates probably from the endoplasmic reticulum. Although at this stage chromosomes are completely uncoiled, relic coil persists throughout the succeeding interphase. The high viscosity which characterizes the metaphase and anaphase decreases during this phase. The centrioles

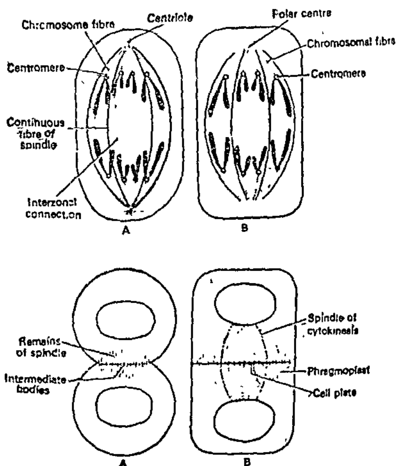


Fig. 80. Diagrams showing the constitution of the spindle during the anaphase and telophase in an animal cell (Left A and A) and in plant cell (Right B and B)

ceases their activity and asters become less conspicuous. The end of the telophase coincides with the division and separation of the cytoplasm of daughter cell. Endoplasmic reticulum also contributes this event. Spindle fibres break down and are absorbed in the cytoplasm. In final stages, the nucleoli reappear at the nuclear organizers, or SAT zones.

Broadly speaking the changes which occur in the chromosomes at telophase can be described as the reverse of those that have taken place at prophase.

Cytokinesis—While these changes are going on in cell, the cytoplasmic division starts as early as the late anaphase—a process which divides a cell into two daughter cells. This process is known

as cytokinesis and considerably differs in plant and animal cells. Cytokinesis in most of the animal cells proceeds by the process of furrowing at the equatorial region. The furrow is accentuated by and deepens until the cell divides. The two daughter cells may be equal in size or they may be quite unequal. In plant cells, there appears a cell plate between the two forming cells in the middle of the spindle. This plate forms the membrane, separating the two cells.

The following table indicates the time taken by each stage in mitosis, in different tissues.

TABLE 5—DURATION OF MITOTIC STAGES IN VARIOUS TISSUES

Tissue	Time noted in minutes			
	Prophase	Metaphase	Anaphase	Telophase
Onion root	71	6·5	2·4	3·8
Pea endo-sperm	40	20·0	12 0	110·0
Grass-hopper neuro-blast cell.	102	13·0	9 0	57 0

Time sequence of mitosis—The speed of mitosis cycle varies enormously. The time of division depends upon the organism, the tissue, the temperature and other environmental, mechanical and chemical factors. The division of neuroblast cells of grasshopper embryo is completed in about 8 hours at 26°C as pointed out by Carlson (1941 to 1954). In bacteria, a culture in the logarithmic phase of growth will divide after every 20 minutes. The division in the microscope of *Tradescantia* take place approximately 16 to 40 minutes at 30°C with 10 to 12 hours elasping between successive division (Fisher 1952).Cohn (1964) pointed out that the mitotic cycle end from 30 minutes to 3 hours in an average cell.

During cytokinesis the distribution of the cytoplasmic components take place. The division of mitochondria and Golgi substance also occur.

CELL CENTRE AND MITOTIC APPARATUS

Cell centre is a cytoplasmic organoid, represented by a single

or double granules, called **centriole** which is generally observable as such in the interphase stage of the cell. During the process of mitosis, it becomes a part of a large and elaborate structure which is called the **mitotic apparatus**. Thus the mitotic apparatus can be defined as "assemblage of structures that constitutes the achromatic figure in the mitosis and includes the *aster* or *astrosphere* that surrounds the centriole and the mitotic spindle." This apparatus can easily be dissolved in alkaline thioglycolate and can be analysed biochemically. The main component seems to be a protein which is low in areomatic aminoacids and containing 2 to 3 percent nucleic acid, the most of which is RNA. The chromosomal DNA is present in negligible quantity.

Centriole—Centriole is lodged in the centrosome and lies just outside the nuclear membrane. Its position is generally fixed for each type of cell. In some cells, it has a tendency to occupy the geometric centre, such as in leucocytes. In general the centriole is pushed back by the nucleus. Structurally under the electron microsocope, each appears as a cylinder of 150 mμ in length, the interior of which is low in density but the wall is electron-dense and contains small rods of tubules of 150 to 200 A° in diameter. These are oriented parallel to the axis. The centrioles appear to be surrounded by a clear zone in the cells undergoing mitosis. This zone is called the **centrosphere**. The two constitute the **centrosome or microcentrum**. It is this centrosphere from which the aster or astrophere radiate during anaphase. During the prophase when the centrioles separate towards the pole, the microcentrum forms an elongated body or bridge, the centrodesmosis from which spindle seems to arises.

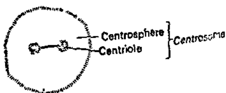


Fig. 81. The centrosome in interphase.

The centriole shows a regular cycle during the cell division. During interphase, they lie at one side of the nuclear membrane. At the start of prophase, one centriole start moving towards the periphery of the nucleus and the other remains stationary. The migratory centriole finally reaches to pole opposite its own by the end of the prophase. Between the two, elements of the spindle appear which soon form a continuous array of spindle fibres between the two poles. Around each centriole aster also develop with the

free ends of the fibres lying in the cytoplasm. During metaphase and anaphase, the centriole remain practically unchanged but in telophase, each divides into two, the two are connected together until next mitotic prophase. As centriole division occurs in advance of mitotic division, it should be considered as the first significant event in the preparation of cell for division.

Spindle—The spindle fibres are present by metaphase stage. The axis of the spindle apparatus is determined by the position of some type of centre. Their origin is probably cytoplasmic rather than nuclear. Although previously it was believed that the nucleoplasm liberated after breakdown of nuclear membrane forms the spindle fibres. But the most compelling evidence for its cytoplasmic origin is that in the animal cell the spindle apparatus develops before the breakdown of the nuclear membrane.

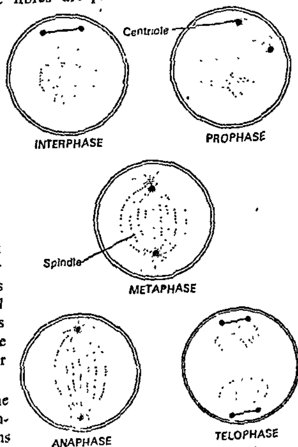


Fig. 82. Centriole cycle during the cell division.

Chemically the spindle fibres are composed of protein chains linked by sulphur and hydrogen bonds. Protein components are mainly acidic and is associated with RNA. The fibres consist of about 90% protein and 5% of RNA. They also contain some lipid, probably as lipoprotein. The sulph-hydryl-group (—SH—) of fibres are present in the prophase but not in anaphase. The disulphide (S—S) linkages maintain fibres integrity when fibres are formed.

Physically the spindle fibres substance is like an elastic gel and

each fibre is filamentous or tubular in structure. A single fibre is 15 m μ in diameter and several filaments are arranged in a bundle to form the fibre as seen with the light microscope. These type of spindle fibres have been recognized between the poles or centriole regions.

1. **Continuous fibres**—Fibres that are continuous from one pole to another.

2. **Chromosomes fibres**—These extend from the centromere of chromosomes to the pole.

3 **Interzonal fibres**—These are present between the centromeres of separating chromatids.

It has been suggested that the movement of chromosomes at anaphase primarily depends on the contraction and expansion of the spindle fibres. When the chromosomal fibres contract, the chromosomes are pulled towards the respective poles. At the same time, the interzonal fibres expand which push the chromosomes to the poles. This however, does not occur in plant cells, owing to the restrictions imposed by the rigid cell wall. Some suggest that some energy is necessary for the movement of chromosomes along the spindle fibres at anaphase. This energy is probably provided by the ATP, though it requires still confirmation.

Significance of Mitosis—Mitosis is a biological system which is significant for many reasons, as it plays an important role in the reproductive devices of the organism. The maintenance of the genetic integrity of the cell population and ultimately of the organism and its descendent depends upon the mechanism of division. Through mitosis the constancy of species is maintained. During mitosis the exact longitudinal division of the chromosomes into chromatids take place and the meticulous distribution of the chromatids to daughter cells, insure that the daughter cells will have the same genetic constitution, qualitatively and quantitatively, as the original cell from which they arose. Linear *heredity* is established, whether it be from cell to cell or organism to organism.

SUMMARY

The cell division comprise two main phases, *i. e.* karyokinesis and cytokinesis. In the former the chromosomes split and become paired structures as chromatids. Each of which finally becomes a chromosome in each of the newly formed cells. Cytokinesis involves the division of cytoplasm.

Mitosis is the characteristic of somatic cells, maintaning the same member of chromosomes as were originally present in the parent cell. The mechanism of nuclear duplication alone is called mitosis. Karyokinesis can be studied under the four sub-stages, *i. e.* prophase, metaphase, anaphase, and telophase. The interphase stage, wrongly called as resting stage is a critical period when the cell prepares itself for the cell division. Before the division sets in, the duplication of DNA and consequently of chromosomes takes place. In the prophase stage the chromosomes appear, each formed of two chromatids, joined at centromere. Spindle starts appearing and the nucleolus starts gradually disappearing. By the end of prophase, the nuclear membrane breaks up. The chromosomes struggle to reach the equator of the spindle and when they have reached the metaphase ends ; the centromere duplicates. During anaphase, the chromatids separate and move apart to the respective poles, caused by the contraction of chromosomal threads and the elongation of the pushing body. The separated chromatids are now called daughter chromosomes. Telophase marks the end of keryokinesis and during this phase the chromosomes undergo decondensation, nucleolus starts appearing and the nuclear membrane is reconstituted. Spindle breaks up and absorbed in the cytoplasm.

The animal and plants mitosis are basically very similar. The animal division deffers from the plant cell division in the presence of centrioles and in the absence of cell plate. The cell plate forms across the middle of the spindle and extends out untill it meets the sides of of the dividing cell.

The factors that control the cell division are little known.

16

MEIOSIS

The evolution of meiotic division was of utmost importance and significance for the development of sexual reproduction in animals and plants. This type of cell division occurs in the cells of testis and ovary. It and the fertilization are the compensating events ; a failure of one or the other causes a breakdown in the orderly system of sexual reproduction. If it occurs after the fertilization (as in most sporozoa, and Ascomycetes and some algae among plants) the adult organism will be an haploid. If the meiosis occurs before the fertilization, during the formation of gametes (as in most of higher animals) the adult organism will be a diploid. In diploid organism, during the process, the normal diploid set of chromosomes in a cell is reduced to a haploid set in each gamete so that when they fuse to form the zygote, the diploid number is restored. This process takes place during the gametogenesis, during the maturation of sex cells. It essentially consists of "two nuclear divisions, which follow each other rapidly while the chromosomes divide only once." The first division involves the separation of chromosomes that were paired during prophase. In this pair one is maternal and other is paternal. Their separation lead to the formation of haploid nuclei. The second division involves the longitudinal separation of chromatids in each of these two haploid nuclei with the result, four haploid nuclei are formed. This is accompanied by the appropriate division of the cytoplasm. The term meiosis was coined by J. B. Farmer (1905) with J. E. Moore and the cells in which the meiosis occur are called the meiocytes.

Induction of meiosis—It is not well known, what is there that initiates the process of meiosis. However, certain physiological changes are attributed essential for the transition of a particular cell from mitotic behaviour to meiotic behaviour. Some state that the meiosis initiating substances are produced by the adjacent somatic tissue. Such somatic tissue includes the non sporogenous tissue, surrounding the megaspore mother cell and which by meiotic

division give rise to megaspores in plants and non germinal tissue in association of the seminiferous tubules, in which the sperms are produced in animals. The nature of this chemical substance is not well known. It may be supposed that it may be hormones or hormonous in nature. Whatever may be the conditions for meiosis in the sexually mature organism,the cell of this type of division is determined during the development of embryo. The meiosis is always under the genetic control, however it is not known that how the genes operate in their control.

Sinha (1950) suggested that RNA : DNA ratio determines whether a mitotic or meiotic division will ensue. According to him a higher RNA : DNA ratio results in mitosis and when this ratio becomes lowerer the meiosis sets in.

THE MEIOTIC CYCLE

Meiosis comprises two nuclear divisions *i. e.* (i) Heterotypic division, and the (ii) Homeotypic division. The **heterotypic division** is also called the **first meiotic division** or **Division I**. During this the diploid parent cell divides into two daughter cells, each having haploid set of chromosomes. The **homeotypic division** or the second **meiotic division** or **Division II** is equatorial in character.The two haploid cells formed as a result of heterotypic division which again divide mitotically into two cells each. Thus from a single parent cell containing 2x-chromosomes (diploid) are formed four daughter cells, each having the x-number of chromosomes (haploid).

HETEROTYPIC DIVISION (DIVISION I)

It consists of four stages like mitosis. It is, however, very difficult to recognize that when one stage ends exactly and another begins. The four stages are :—

1. Prophase I.
2. Metaphase I.
3. Anaphase I.
4. Telophase I.

1. PROPHASE I—In the premeiotic interphase, the chromosomes also have the same relic coils as in the premitotic interphase. In the interphase stage, the chromosomes exhibit very little or no movement but as the prophase I starts, they move in several ways and the nucleus starts increasing in volume and this increase is much than the mitotic increase to some extent. This is due to an increase in hydration. To this stage Nebel and Ruttle (1936) has called

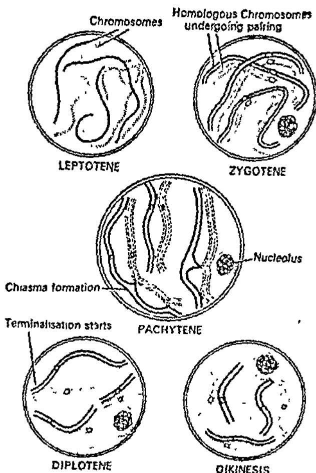


Fig 83. Various stages of prophase in heterotypic division.

"premeiotic spiral prophase". Prophase I is of extremely long duration as compared to mitotic prophase, and comprises mainly five substages, *i. e.* Leptotene or Leptonema, Zygotene or Zygonema, Pachyene or Pachynema, Diplotene or Diplonema, and Diakinesis.

A. Leptotene—This substage does not differ much from the earliest prophase stage of mitosis except that the cells and the nuclei are generally longer than those of the surrounding somatic tissue. This substage is characterised by the following main features. Volume of the nucleus increases with the result it assumes a larger shape. The chromosomes become apparent as long uncoiled and slender filaments which are well separated from each other. These filaments correspond to the chromonema of the anaphase of mitotic division. The arrangement of the filaments (chromosomes) is often irregular but they may show a definite orientation and a tendency to be

polarized directing one or both ends towards the nuclear membrane, commonly towards the point where the centrosome is to be located. They are arranged parallel, well separated but at a point of polarization they are close together ; the distal ends spray out in the nuclear cavity. This peculiar arrangement has sometimes been referred as "bonquet". The chromosomes develope a number of small coils, which vary in their degree of condensation. The tightest coils are known as chromomeres because of their greater density, *i. e.* chromomeres start appearing. The others are called the major coils, which gradually grow in diameter as prophase proceeds. The chromomeres are constant in number, size, and position. Belling (1931) estimated about 1500 to 2500 chromomeres in the whole chromosome set in *Lilium*.

In some cells, DNA and histone synthesis and chromosomes duplication occur in this stage while in others it occurs in later sub-stage of prophase I. In most cells this duplication of chromosomes becomes completed by the end of the next substage, the zygotene. In general it is assumed that the chromosomes are duplicated by the end of leptotene, so that it consists of two chromatids, joined by centromeres. The nucleolus is well marked. In some cells it is small first than becomes enlarged during leptotene and zygotene. It is believed that the increased synthesis of RNA and proteins in early prophase I results in the increasement of nucleolar size.

B. Zygotene—The end of leptonema finds the chromosomes shorter in length and wider in diameter because of the gradual increase in the diameter of the earlier formed spirals. The following are the important events which commence and end during this substage.

The pairing or synapsis of homologous chromosomes starts. The two similar or homologous chromosomes pair lengthwise with each other in a pattern characteristic of the species. Out of the two synaptic chromosome, one is of paternal origin while the other is of maternal. These are called bivalents. Since each chromosome is formed of two chromatids, each pair of homologous chromosomes consists of four chromatids and this is sometimes referred as tetrad. The synapsis occur part by part. The polarization possibly facilitates the initial union of the homologous chromosomes.

The coiling of the chromosomes continues to produce a

marked condensation. The chromosomes become shorter and thicker as the major coils of each chromatid increase in diameter. These coils are larger than the somatic coils of the mitosis. In addition to major coils, there is a relational coiling of the chromatids. Nucleolus increases in size and centrioles move apart initiating the spindle formation.

C. **Pachytene**—*It generally begins when the pairing of chromosomes ends.* It represents one of the longer substage of prophase I and is characterised by the following important events.

When the pairing has completed, the longitudinal contraction starts with the result the chromosomes become short and coarser. Within the major coils, and at right angles to them smaller minor coils appear. At the height of pachytene, the pairing chromosomes twist about one another as relational coils and each soon longitudinally splits, if no splitting had occurred. This coiling is further complicated by a coiling of two chromatids of each chromosome around each other. This coiling puts the chromosomes under considerable strain. This means that at this stage, each pachytene element consists of four chromatids. The chromatids of each homologue are called **sister chromatids**. Formerly this element of four chromatids was called a **tetrad**—a name which has now been replaced by **bivalent**. When the chromosomes have longitudinally splitted, transverse breaks occur at the same level in two of the adjacent homologous chromatids Soon both segments of the chromatids interchange and fuse together with those of homologues. In this process, which is called **crossing over**, portions of the two chromatids with their genes are interchanged and two remain intact The new chromatids thus interchanged will be mixed.

After this interchange, the parts begin to separate, repelling each other. However this separation is not at all complete since the homologous chromosomes remain joined at their point of interchange. This point where the two are joined is called the **chiasma** (Pl. chiasmata). This chiasma is an *immediate consequence* of crossing over and are found in all plants and animals with few exceptions (Eg. Diptera males and female silkworm). The number of chiasmata is variable but for one bivalent atleast one chiasma is found. Bivalents with 2, 3, and 4 chiasmata are rare. The highest number of chiasmata has been observed in the long chromosome of the broad bean, *Vicia faba*, where there are probably 12 chiasmata in a bivalant. The average number of chiasmata is known as chiasma

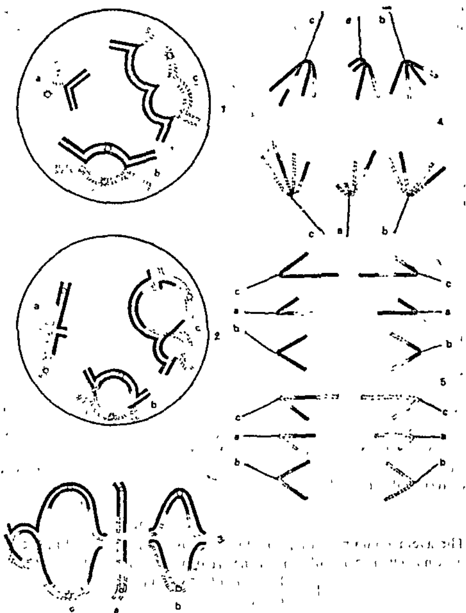


Fig. 84. Diagrams showing the genetic consequence of the meiosis of three pairs and chromosomes with (a) one chiasma, (b) two chiasmata and (c) three chiasmata ; 1-Diplotone ; 2-Advanced diplotene showing terminalization ; 3-Metaphase I ; 4-Anaphase I ; 5-Anaphase II. [Broken lines represent the maternal. Circle represents the centromere].

frequency. This number seems to depend upon the length of the chromosome. Short achrocentric chromosomes have a greater number of chiasmata than metacentric chromosomes. In small chromosomes, chiasmata are localized at the distal ends. Nucleolus remains as such being somewhat larger than the preceeding substage.

D. Diplotēne—During this substage, the chromosomes exhibit following changes.

During pachytene, the paired chromosomes do not show the tendency to fall apart. This tendency become apparent during diplotene, so much so that the members of each pair in some organisms become completely dissociated to form univalents ; where they do not dissociate completely, it is because of the one or more chiasmata wnich bind them together. As the chromosomes separate, they open out to form the loops and nodes characteristic of diplotene ; the chiasmata are the nodal regions.

At the end of diplotene, the chiasmata begin to move along the length of chromosome from the centromere. This displacement of chiasmata has been referred as **terminalization** by Darlington (1930). This rotation is well marked in bivalent having one chiasma. The Fig. 84 shows the arms of a bivalent exicuting a movement comprising a rotation of 180 degrees until they acquire the form of a cross. If two chiasmata are there in a bivalent, the openings continue to widen so as to form a ring. In cases where chiasmata are more, the rotation, gives rise to figure of a chain of links, each perpendicular to next. When the terminalization of chiasmata is complete, the homologues are kept in contact by terminal chiasmata. The degree of terminalization is generally expressed as a **coefficient of terminalization (T).**

$$T = \frac{\text{Number of terminal chiasmata}}{\text{Total number of chiasmata}}$$

The average number of chiasmata in a bivalent or in all bivalents of a nucleus is generally called the **frequency of chiasmata (Fq)**

$$Fq = \frac{\text{Total number of chiasmata}}{\text{Total number of bivalents}}$$

According to Darlington there are two kinds of repelling forces operating at diplotene. One with less effective electro-negative charge concentrated on the surface of the chromosome throughout its length and the other with a special charge localized at the centromere. The first forces the chromosomes apart while the other causes distal movement of the chiasmata to the ends. Swanson *(1957)*

does not agree what Darlington proposed for terminalization and suggested that it occurs by despiralization of the chromosomes. According to him the change from a condition of many small coils to one with fewer and larger gyres develop mechanical tension which is strong enough to cause chiasmata to slide along the chromosomes.

E. Diakinesis—The transition from diplotene to the diakinesis takes place gradually. Following events mark the diakinesis.

Bivalents become very short and thick, They stain quite deeply. The two chromatids of each chromosome are very close to each other so that the individual chromatids are not identifiable. Terminalization may be completed in this substage, if had not been completed in diplotene, and the number of chiasmata diminishes The bivalents migrate to the periphery of nucleus and are apart from each other. The nucleolus begin to disappear and is no longer visible at the end of the diakinesis. The last event includes the disruption and dispersal of the nuclear membrane releasing the chromosomes into the cytoplasm of the cell. At this time the spindle fibres have organised establishing the poles of the cells The pole determines the axis of orientation of the chromosomes in metaphase I.

2. METAPHASE I—It is characterised by the complete disappearance of the nuclear membrane and the formation of spindle. The chromosomes move to the equator of the cell. The stage in which the movement of chromosomes is recorded has also been named as prometaphase of meiosis. The bivalent chromosomes arrange themselves in the equatorial plane with their two centromeres directed towards the opposite poles and their arms towards the equator. This is quite different from that in mitotic metaphase where the centromeres lie equatorially and the arms are directed towards the poles. Moreover in mitosis there are only one centromere in the beginning which later divides ; in meiosis there are two centromeres from the beginning. The repulsion of the two centromeres is accentuated and the chromosomes are now ready to divide. If the bivalent is long, a series of annular apertures are formed between the chiasmata in paires, alternating perpendicularly among themselves. If the chromosomes are short, there is a single aperture. In some cases, there is a third splitting by which the bivalents comprise a double chromonema in each of their chromatids. In such cases, each bivalent is formed of eight filaments instead of four.

The behaviour shown by chromosomes in metaphase I is a

significant one. When they are oriented as bivalents at the equat of the cell, the segregation of the paired (factors genes) occurs. La of independent assortment is also illustrated by the prophase I ar metaphase I. Two pair of factors (genes) are involved. T chromosomes are oriented in a random fashion.

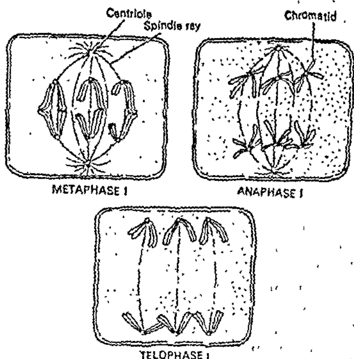


Fig. 85. Further stages in heterotypic division.

3. ANAPHASE I—The movement of the daughter chromatids of each homologue, united by centromeres towards their respective poles constitute the anaphase I. The tetrads become separated into dyads having two chromatids each. The two chromatids widen out from each other except at the centromere assuming V-shaped form. The shapes however differ and depend on the position of centromeres. The dyads when separate, they have a different composition from that of originals. Two of their chromatids are mixed ; the other two maintain their inital nature. Each centromere is directed towards a different pole ; the maternal to one and the paternal to the other pole. Thus each group at anaphase is formed of haploid number of chromosomes instead of diploid. The poleward movement of the dyads looses the relative influence of chiasmata and finally free the separating chromosomes.

4. TELOPHASE I—As soon as the anaphasic groups arrive at their respective poles, the telophase sets in. In this stage following changes take place.

The chromosomes elongate by lessening their coils. The nucleolus again reappears. The nuclear membrane appears around each group of chromosomes. Cytokinesis may or may not occur and so the product of the first meiotic division may be two cells or two nuclei with a common cytoplasm. In the latter case, the nuclei will be separated by a plasma membrane at the end of the second miotic division.

In *Trillium* and certain members of the odonata, the anaphase indirectly passes into prophase II, omitting telophase I and interphase. The cells retain the coiling of the chromosomes and persists infact throughout the interphase.

Interkinesis—After telophase, two haploid daughter nuclei or cells sometimes undergo typical resting stage as in mitosis. This intervening stage between first telophase and the beginning of second prophase is termed as **interphase** or **interkinesis**. This stage is either very short or may be entirely absent.

HOMOETYPIC DIVISION (DIVISION II)

PROPHASE II—This stage superficially resembles the nucleus of the mitosis prophase with the following exceptions.

At the early prophase II, the two chromatids of each dyad look like X, since they are conjoined by a common centromere. The four arms are widely separated. There is no relational coiling. The X-shaped dyads are quite longer than at telophase. The chromonemata are still not completely coiled. The genetic constitution of the two chromatids of each dyad depends upon the kind and number of cross overs which took place in the I meiotic prophase. If there is no crossing over, the dyads would consist of two identical sister chromatids of either paternal or maternal origin. At the end of the prophase II the nucleolus disappears. The nuclear membrane disappears and the acromatic figure is developed.

METAPHASE II—It is of very short duration like mitotic metaphase. The chromosomes become arranged on the equatorial plane. The centromeres lie along the equator and the arms extended outwards. The centromeres divide and the daughter chromatids direct themselves towards the opposite poles.

ANAPHASE II—This substage begins when the chromatids

or daughter chromosomes with their individual centromere move towards the opposite poles. The chromatids are now short and thick as has been observed in anaphase I of metotic division. They are much like the anaphase chromatids of mitosis.

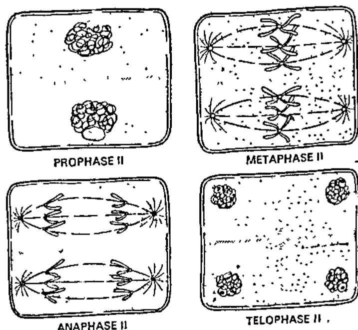


Fig. 86. Stages in homoetypic division.

TELOPHASE II—At each pole, nuclear membrane develops and surrounds the daughter group of chromosomes present. Nucleolus reappears. As a result of cytokinesis, two cells are formed from each haploid cell.

Thus we saw, that by meiosis four nuclei or cells develop or produced from one germinal cells, each having the haploid number of chromosomes. The two nuclei contain the paternal set and their centromeres, and the remaining two have maternal set and their centromeres.

MITOTIC AND MEIOTIC DUPLICATION OF CHROMOSOMES AND CHROMOSOMAL DNA

Cytological observations of prophase mitotic and meiotic chromosomes have shown them to have undergone duplication by the time they are visible under the microscope. Thus it can be suggested that the actual duplication of the chromosomes occurs

before prophase. It is now possible to detect the chromosomal DNA either by using the specific stain or by the use of the radioactive isotopes. With this it is easy to follow the chromosome duplication by following the chromosomal DNA. As a series of experiments done by the eminent cytologist J H. Taylor, it is now possible to suggest the appropriate mood of duplication for both mitotic and meiotic DNA in the chromosomes of higher organisms that closely parallel to the lower organism. The mitotic duplication have been studied in seedling of the bean, *Vicia faba.* As to have a complete understanding of chromosomal duplication, *Vicia* seedlings were grown in solutions of radioactive thymidine, a molecule that contains one of the pyrimidines of DNA. The thymidine was found to be taken up by the roots of the seedlings and then incorporated into the DNA of the chromosomes of root tip cells. In this way the seedlings were grown for a long time in the presence of radioactive thymidine, until and unless the chromosome undergo duplication. After this the roots were washed and transferred to solutions without radioactive thymidine. The non radioactive solutions, however, contained colchicine, a drug that blocks spindle formation and thus nuclear division, but does not block chromosome duplication.

When the chromosomes were examined after duplication in the radioactive thymidine, it was noted that each pair of the daughter chromosomes was labled with the isotope. After a second duplication, but however, in the absence of the isotope, it was noted that among the daughter chromosomes one was labled with the isotope and the other was not. In other words, it may be that a labeled chromosome gave rise to one labeled and one unlabeled chromosome.This is really based on the assumption that the chromosome contains at least two DNA units. However, in the formation of the daughter chromosomes these units separate and each unit duplicates. When this duplication occurs in the presence of radioactive thymidine, the two daughter chromosomes will be labeled. Further a second duplication in the absence of isotope will thus lead to one labeled and one unlabeled chromosome. This result is in accordance with the structure of DNA and its mode of duplication as was proposed by Watson and Crick.

In the same way, the labeled thymidine was also used by Taylor to investigate the meiotic duplication of chromosomes in

males of the grasshopper, *Romala microptera*. The isotope was injected into the animal and testes were removed from different animals at various time intervals. The distribution of the label in the meiotic chromosomes during spermatogenesis was found to be similar to that found for mitosis. In other words, the DNA of the meiotic chromosomes also duplicates in the semiconservative fashion. Thus the significant facts indicated by Taylor about the chromosome duplication, are in accord with the observations derived from density-gradient centrifugation.

BEHAVIOUR OF CHROMOSOMES AT PROPHASE I

1. Synapsis of chromosomes—

There is no adequate explanation as to how the homologous chromosomes undergo pairing or synapsis in meiotic prophase. In early prophase, the chromosomes lie quite apart from each other, yet they manage to come to lie side by side for their entire length by the end of zygotene. This process of pairing or synapsis when once started proceed in zipper-like fashion along the entire length of each chromosomes pair, until it is complete and there are no unpaired regions left. This pairing is a very intimate one which is not merely between the homologous chromosomes but always between strictly homologous regions. The synapsis occurs at zygotene and in majority of meiotic cells, all the chromosomes become entirely synapsed by the end of pachytene, the next substage.

There are two major theories to explain the cause of pairing.

A. **Precocity theory**—According to Darlington, the leptotene chromosomes are not divided into chromatids and, therefore, the homologous chromosomes pair because of their singleness. In the light of evidences that DNA synthesis and chromosome duplication take place as early as premeiotic telophase in some organisms, this theory is not well substantiated.

B. **Retardation theory**—This theory was proposed by Sax (1932) and mainly based upon a thesis of retardation of cellular metabolism during meiotic prophase. According to this theory the extended time period of prophase I allows the uncoiling of the relic coils of the preceding interphase and telophase so that there is complete uncoiling of the chromosomes. As a result, the part by part pairing of homologous chromosomes in zygotene is greatly enhanced. This theory also does not explain the things properly.

As regards the mechanism of pairing, a lot of explanations

have been given by various workers but all lack one or the other things. However, it is generally believed that there exists some long-range attractive forces between the homologous chromosomes which attract each other to effect their pairing (Muller, 1941). These attractive forces are due to the inherent vibrational frequencies in the chromosomes, with different regions having perhaps different frequencies; the similar vibrational frequencies of homologous chromosomes attract each other so that the two like chromosomes move towards one other (Faberge, 1942). White (1954) has well said, "we accordingly have to visualize, not merely a general attraction between homologous chromosomes but many thousands of different forces of attraction (presumably as many as there are pairs of genes)". Accordingly, each locus would produce a characteristic pairing force different from that of all other loci.

According to Darlington (1935), the pairing can be of three types:

(a) **Proterminal**—When the two homologous chromosomes start pairing by their ends and progress gradually towards the centromere region.

(b) **Procentric**—When the synapsis starts near the centromeres and proceeds towards the ends of the chromosomes.

(c) **Intermediate**—When the pairing is random and occurs simultaneously at various places along the length of the chromosomes. This is also called the random synapsis.

2. Mechanism of crossing over

The phenomenon of crossing over is yet another cytological problem, particularly its origin and the cause. Darlington (1937) [illegible] theory of meiosis suggests a mechan- [illegible] enon. This theory suggests that the [illegible] begins before the chromosomes have reduplicated. Homologous chromosomes pair because of their singleness. The paired (undivided) chromosomes coil relationally around one another so that the direction of the relational coiling is opposite to that of the internal coiling. Thus the two types of coils are in physical equilibrium. Now the division of chromatids takes place and this upsets the said equilibrium and causes the torsion of the chromatids. This results in break in one chromatid which is followed by a break in a second chromatid at exactly [illegible] same point. These broken ends move apart, rotate and thus [illegible] occurs, giving rise to crossing over, amongst the chromatids.

torsion hypothesis has been criticized by various authors (Sax, 1936); the main objection is that the chromosomes are duplicated before synapsis.

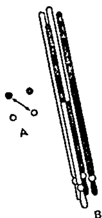
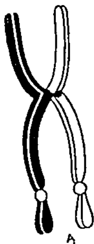


Fig. 87. Diagrams showing the process of crossing over.



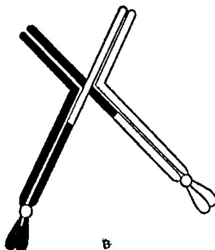


Fig. 88. Diagrams showing the crossing over (A) and chiasma formation (B).

Modern studies point out that there are atleast two mechanisms which results in crossing over. Firstly the reciprocal exchange of chromatids occurs during or prior to pachytene, when four chromatids are observable. Side by side, some recombination positively occurs at the time of DNA synthesis before zygotene pairing. In doing so a copy choice mechanism has been suggested to occur, *i. e.* a part of new DNA helix being synthesized may copy a non sister helix rather than a sister helix, to produce a recombination in a very short segment. When this copy-choice crossing over occurs, the crossing over amongst the sister chromatids may occur to produce the similar genetic variation.

3. **Chiasma formation**

Chiasmata (*Sing* : chiasma) have observed during prophase I in most organisms. Their formation is necessary for orderly segregation at meiosis. Two theories have been put forward to explain its formation.

(a) **Classical theory**—This theory was ... rward by Sax (1932). This suggests no breakage of chr... re the formation of chiasma. Therefore ... four th... ds) at

consequently unaltered. According to this theory a chromatid of paternal origin actually crosses over one of maternal origin in such a way that on one side of the chiasma a paternal chromatid is paired with a paternal and a maternal with maternal. On the other side of chiasma a paternal is paired with maternal and a maternal with

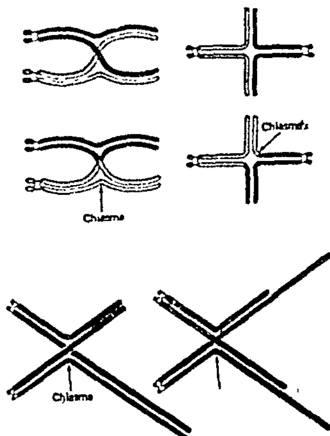


Fig. 89. Diagrams showing the difference between the classical (UPPER) and chiasma-type (MIDDLE) theories of chiasma formation. [Towards the left interpretation has been shown and towards right the same after rotation has been shown; Maternal chrosome are shown black; paternal are shown stippled]. BOTTOM—Figs. provide proof that chiasmatype is correct. An unequal bivalent with a single chiasma as actually found (Left) and what would happen in such a bivalent if the classical theory is correct, not found. (Right)

paternal. In short chiasma formation leads to a genetic cross over through breakage of the chromatids when they repel each other. The crossing over follows the chiasma formation. Recent studies refute this theory.

(b) **Partial chiasma type theory**—This theory was proposed by Janssen (1909) and Darlington (1932) and is beleived to be the most widely accepted view as regards the formation of chiasma. They stated that the chiasma is derived from the breakage and reunion of two homologous chromatids, out of the four in such a way as to produce an X. A genetic cross over has preceded the appearance of chiasma and give rise to it. There are, however, some cases (*Drosophila*) where chiasmata have nothing to do with the crossing over. According to this theory a paternal chromatid is associated with another paternal one and a maternal with another maternal one on each side of the chiasma.

4. Genetic map

If in the same chromosome, the two genes are located very close to each other, the chances of crossing over are meagre. If a cross over between the two genes occurs, two of the nuclei will have parental combination and the other two nuclei will have new combinations or recombinations. It has been suggested that farther apart the two genes are in the chromosome, the greater will be the frequency with which a chiasma or crossing over will be produced between them consequently, the recombinations will be more frequent between the two of the nuclei from each meiotic process. Such data are used in constructing genetic maps of chromosomes. The number of units between the two genes on a map is given by the percentage of recombinations among the products of a large number of meiosis. In cases, where the two genes are very close to each other in the chromosome, the percentage of recombination is low.

In each chiasma only two chromatids out of four interchange. Thus the percentage of recombinations will be equal to half the average frequency of chiasmata.

Multiple exchanges

As has already been stated that when a chiasma is formed only two chromatids, out of four (50%) cross over. However, more than one cross over may occur in a chromosome. Each chiasma occurs independently, as such if two chiasmata occur, there may be four possible classes of resulting combinations. In the later case two,

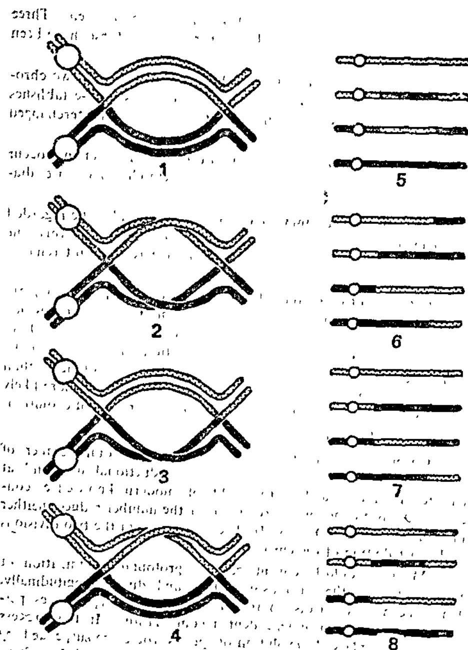


Fig. 90. Different relationship between two successive chiasmata (1, 2, 3, 4) and the products of the double exchange (5, 6, 7, 8). 2—Complementary pairs of chiasmata (four strands exchanged; 3, 7, and 4, 8—diagonal pairs of chiasmata (three strands exchanged.)

three or even four chromatids of a bivalent may be involved. Three types of *multiple exchange* have been observed and these have been depicted the Fig. 90.

(a) **Reciprocal chiasmata**—In this type the same two chromatids are involved in both events; the second chiasma re-establishes the original configuration. This results in two non interchanged chromatids and two with double crossover.

(b) **Complementary chiasmata**—In this type inter-change occur in all the four chromatids; the third and fourth chiasmata are diagonal ones.

(c) **Non compensatory chiasmata**—This can also be regarded as diagonal without three chromatids taking part. In this one chromatid remains without interchange ; two have one interchange and one has double interchange.

5 **Significance of crossover**—The crossing over provides an opprtunity to the genes so that they can shuffle themselves—a thing which makes sexual reproduction an advantageous process. This allows a practically infinite variety of gene combination and of individuals. Some of these are more likely to be suited to the environment than others and so under the influence of natural selection are more likely to survive to become the positive factors in the process of evolution.

Significance of meiosis—

Generally it is said that during meiosis, the total number of chromosomes is "reduced" as a result of "reductional division" at Division I. But actually in the light of modern knowledge concerning the phenomenon of crossing over the number reduces neither in Division I nor in Division II. The total result of the two divisions is the distribution of the chromosomes.

Meiosis should be considered as a profound *modification* of mitosis at which the chromosomes pair and divide longitudinally. It is a mechanism for distributing the heredity units or genes permitting their random independent recombination. If this process does not take place the evolution of species would *be suspended* by unalterable chromosomes and the living nature would have been devoid of its characteristic diversity.

Genetic significance of Meiosis—Gregor Mendel proposed two laws of inheritance. The first law states that, "*the paired factors responsible for a given characteristic segregate into the gametes and are recombined at fertilization.*" The second law states that, "*the*

segregation of one pair of factors Occurs independently of the segregation of a second pair of factors." These laws are based upon the behaviour of the chromosomes during meiosis, though Mendel himself was not aware of the actual mechanism involved. The relationship between the two laws and the meiotic events of the chromosomes are shown in the Fig. 91.

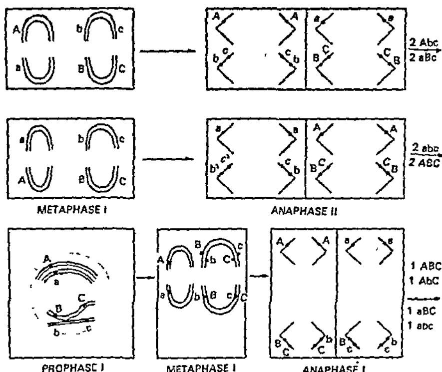
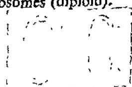


Fig. 91. Genetic aspect of meiosis.

TABLE 6—COMPARISON OF MEIOSIS AND MITOSIS

Meiosis	Mitosis
1. It always occurs in the germinal cells of the gonads (testes and ovaries) particularly during maturation of gametes.	1. It occurs in all the cells of the body, *i. e.* somatic and germinal cells. However during the maturation of the later, it does not occur.
2. The whole process is completed in two sequence with the result four daughter cells or	2. The whole process is completed in one sequence. By mitosis only two daughter cells

Meiosis	Mitosis
nuclei are produced from one germ mother cell. The first division is the reduction division while the second division is the simple mitosis.	or nuclei are produced from the mother cell. 
3. Daughter cells are not similar to that of parent one as crossing over takes place and the chromosomal number is reduced to half in each daughter cell, *i. e.* the resultant cells or nuclei are *haploid*.	3. Daughter cells produced are similar to the parent cell as each possesses the same number of chromosomes (diploid).
4. **Prophase**	
(i) The prophase is of long duration and measured in days.	(i) It is comparatively of short duration.
(ii) There are two phases of prophase, *i. e.* first and second. The first being the long and is subdivided into five substages, *i. e.* leptotene, zygotene, pachytene, diplotene, and *diakinesis*.	(ii) There is only one phase of prophase. *It is of* shorter duration *and is* also subdivided *into* substages.
(iii) The chromosomes are single structures in the beginning and are granular in nature.	(iii) The chromosomes are double structures and are formed of two *chromatids*.
(iv) Nucleus increase in size sufficiently.	(iv) Nucleus increases *less in* size.
(v) Synapsis in the homologous chromosomes takes place.	(v) No synapsis occurs.
(vi) Chromatids of one chromosome exchange with the chromatids of second	(vi) There is no chiasma formation and the *crossing* over does not take place.

Meiosis	Mitosis
chromosome during the process of diplotene. This process of chiasma formation and crossing over occurs during the process.	
(vii) Process of coiling is well marked.	(vii) Process of coiling is not well marked.
(viii) Nucleolus and nucleus membranes disappear in the late prophase and the centriole form the amphiaster.	(viii) Same.
5. Metaphase	
(i) The centromeres lie towards the poles of cell and their arms are directed towards the equator of the cell.	(i) Arrangement is just reverse, *i. e.* centromere lies towards the equator and the arms are directed towards the poles.
(ii) The centromeres undergo no division. They are double from the begining.	(ii) In the beginning of metaphase, there is one centromere. This later on divides to become double.
6. Anaphase	
(i) The chromosomes are very short and thick.	(i) The chromosomes are comparatively less thick and short.
(ii) When the homologous paternal and maternal chromosomes separate, they have different composition from that of original or the daughter chromosomes are haviug different composition than that of original ones,	(ii) The daughter chromosomes are similar to the original one in composition.

Meiosis	Mitosis
7. **Telophase**	
(i) Cytokinesis is not necessary to occur in telophase I but it normally extends to second meiotic division.	(i) Cytokinesis always takes place after each division.

SUMMARY

There are two essential features of sexual reproductions First, haploid cells are produced by meiosis and secondly two haploid cells fuse to form a new diploid individual.

The special type of division that take place in certain diploid cells to produce haploid cells is known as meiosis. When a cell undergoes meiosis, each chromosome pairs with its opposite member, *i. e.* synapsis of homologous chromosomes takes place. Soon each member of the pair duplicates itself and become formed of two chromatids. Thus at this time each pair of chromosomes comprise four chromatids; the stage being referred as tetrad. Chromati [illegible] exchange with the chromati[illegible] over and [illegible] of prophase I. After the tetrad formation, the nuclear membrane and nucleolus disappear just as in mitosis. Spindle is formed. The two members of the each chromosome pair separate from each other. After the chromosome pairs separate, the cell divides into two new cells. This is the first meiotic division which is also [illegible] Soon it is followed by the [illegible] s similar to that of mitosis. [illegible] formed from one. Each [illegible] has one chromosome for [illegible] the original diploid cell. Each of these haploid [illegible]

During metaphase [illegible] towards the poles of [illegible] towards the equator [illegible] undergo no division [illegible] the begining. When [illegible] nal chromosomes sep[illegible] tions from that of original,

17

GAMETOGENESIS

In the multicellular animals, there are two kinds of gametes which are quite unlike in the vertebrates. The female gamete or ovum is invariably larger than the male gamete or spermatozoon and is immobile whereas the other is active and varies greatly in size and constitution. They are produced in the gonads (ovaries or testes) from the germinal cells prerent therein. The sperm with few exceptions is flagellate,and swims actively so as to reach the inactive ovum with which it fuses to form zygote. The process by which the gametes or germ cells are produced from the germinal cells of the gonads is known as the gemetogenesis.If by this process sperms are produced the process is called the spermatogenesis and if this results in the formation of ovum, the term oogenesis is applied. The process of gametogenesis is quite complicated as the germinal cells are diploid containing double set of chromosomes and the gametes which are produced, are haploid containing half the number of chromosomes, found in the germinal cells. It means, during the process the chromosomal number is reduced to half in the gametes and this involves the process of meiosis.

SPERMATOGENESIS

The formation of sperm in animal is very complicated process and takes place in two stages. The first stage includes the formation of spermatids by meiosis and the second stage includes the metamorphosis of spermatids into spermatozoa. The process of formation of spermatids comprise the morphological and cytological changes in the meiotic products.Sperms are always produced in the seminiferous tubules of the testes of the male animal. The spermatogenesis occurs in two stages.

1. Formation of spermatids.
2. Metamorphosis of spermatids.

1. Formation of spermatids—The spermatids are always formed from spermatogonial cells of testes. These cell are diploid in the

number of chromosomes. The whole process of spermatid formation takes place in three stages :—

1. Phase of multiplication.
2. Phase of growth.
3. Phase of maturation.

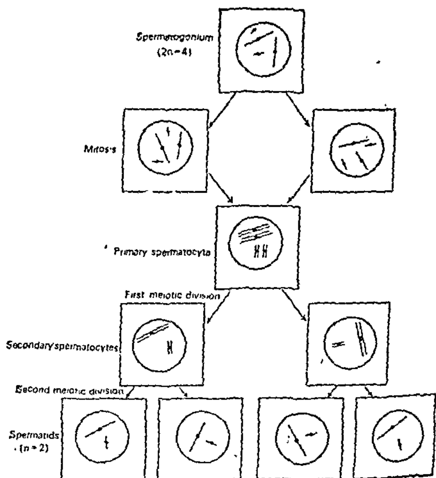


Fig. 92. Spermatogenesis.

Phase of multiplication—All the cells of the germinal epithelium of seminiferous tubules of the testes can potentially develop into spermatozoa, but all do not do so, but only the primordial germ cells do so. Some of the germinal cells (primordial cells) become modified for spermatogenesis and are called as primary germ cells, while other cells function as nutritive cells. These nutritive cells are called

as **Sertoli cells** in the case of mammals The primary germ cell undergoes repeated divisions, all mitotically, and form a large number of **spermatogonia.** Each spermatogonium is diploid.

Phase of growth—As the mitotic division of primary germ cell stops, each spermatogonium enlarges in size and absorbs the nutrition from the side cells. The large sized cells are known as **spermatocytes.** The nucleus of spermatocyte become much larger rather than the normal nucleus. During the growth period, pairing and splitting of chromosomes (like meiosis) take place. Due to this pairing and splitting tatrads are formed. Linkage and crossing over also take place in this phase. In general, this may be called as prophase I of meiotic division.

Phase of maturation—The growth phase is followed by the maturation phase. In this stage, two successive cell divisions take place. The first being the meiotic, during which the number of chromosomes becomes half (haploid) rather than diploid. If a normal diploid number of chromosomes in any of the animal is 2n, each spermatazoon by the reduction process (meiotic division) will have only n chromosomes.Thus the first reduction division or meiotic division result in the formation of **secondary spermatocytes,** each containing n number of chromosomes Each secondary spermatocyte now divides mitotically. With the result of this division **spermatids** are formed. Thus from one germ cell, four spermatids are formed. They do not divide further and undergo metamorphosis to give rise the sperms.

Metamorphosis of spermatids or Spermiogenesis—The haploid spermatid is a typical cell containing nucleus and cytoplasm. The cytoplasm contains mitochondria, centriole and a dictyosome which may be homologous to Golgi complex. The following changes subsequently take place during spermiogenesis.

1. The developing spermatids increase in size (lengthwise) and loose most of the cytoplasm.

2. Centriole divides into two daughter centrioles. The distal centriole forms the main axis of the tail of sperm while the proximal centriole becomes attached to nuclear membrane. The axis or the **axial filament** is made up of a complex of fibrils throughout its length Subsequently, the distal centriole becomes the basal body of the tail or flagellum of the mature sperm.

3. The axial filament is surrounded by a fibre-coat just behind

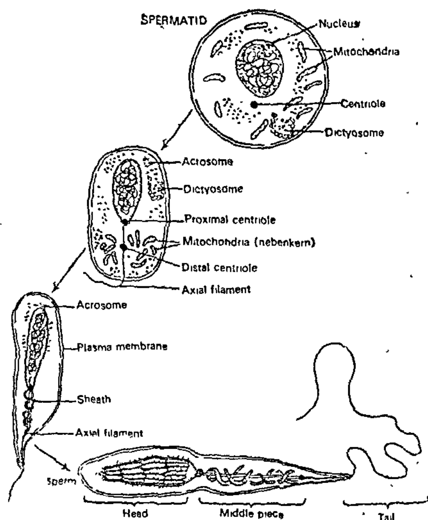


Fig 93. Maturation of spermatid.

the region of sheath with a plasma membrane outside. But near the tail end, it is surrounded only by membrane.

4. The mitochondria elements become oriented at the side of axial filament as two densely packed bodies. They form the spiral sheath around the filament. This sheath is known as nebenkern. The number of the folding of sheath vary in different animals, for example there are 12 turns in human spermatozoon, and 115 in bat spermatozoon. The spiral sheath provides the energy to the sperm for movement towards the eggs and this energy generally resides as adenosine triphosphate (ATP) in mitochondria.

6. The **Golgi complex** also shows some changes and forms a single large body, the **acroblast**, which give rise to another element called **acrosome** which covers the nucleus.

The developing sperm lengthens and looses most of its cytoplasm. The plasma membrane, however, remains as an envelop around the entire mature sperm including the tail. The mature spermatozoon or male gamete, consists typically the three main parts, the **head, the middle piece and the tail.** The head consists of an elongated nucleus, the acrosome, a surface membrane (probably cytoplasmic in origin), and occasionly other elements. The middle piece is composed of centriole, an axial filament and a sheath. The tail which is considerably longer, composed mainly of axial filament.

OOGENESIS

Oogenesis occurs in the ovary of the female animals. The three stages of division are similar as in spermatogenesis. They are :—

1. Phase of multiplication.
2. Phase of growth.
3. Phase of maturation.

1. **Multiplication phase**—In this phase, the **primary germinal cells** of ovary with diploid number of chromosomes, divide several times mitotically so as to form a large number of daughter cells. These daughter cells are known as **oogonia.**

2. **Phase of growth**—During this stage the oogonium increases in size with the enlargement of the nucleus. Synapsis, crossing over, and other cytological changes occur as in the spermatogenesis. After these changes the oogonium is called as **primary oocyte.**

3. **Phase of maturation**—The primary oocyte undergoes first meiotic division. This division is generally called as the **first maturation division.** As a result of this the two daughter cells are formed. Out of these two cells, one cell is large called the **secondary oocyte** and the other is smaller called **first polar body or polocyte.** The secondary oocyte again undergoes the **second maturation division.** This division is the mitotic division and two cells are formed from each secondary oocyte. The larger cell is known as **ootid** and smaller one is known as **second polocyte or second polar body.** The first polar body may or may not divide to form two polar bodies. Generally these polar bodies disintegrate later on. In this way the product of whole process of oogensis, is the formation of single large

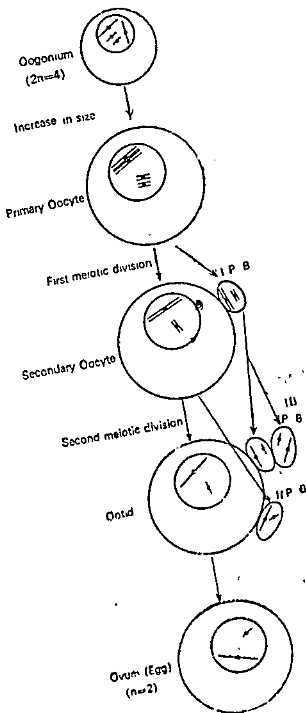


Fig. 94. Oogenesis.

haploid egg and two or three polar bodies (polocytes) found on the periphery or egg.

Chromatin behaviour during Somatic and Meiotic mitosis—As the muturation behaviour of the chromatin components in the spermatocyte and oocyte are similar, so it is better to deal it common. As the prophase condition of the nucleus is initiated, and odd type of the behaviour of the chromosomes become evident—a behaviour which is entirely absent from ordainary somatic mitosis. In the meiotic mitosis, the homologous pairs or mates begin to show an attraction for each other and they form an intimate association. This association is called synapsis. As a result, the two homologous chromosomes appears as one structure. While the homologous chromosomes are intimately associated, each mate reproduces itself longitudinally. Hence each bivalent chromosomes becomes transformed into four potential chromosome, each one of which called a chromatid. This group of chromatids, collectively called a tetrad chromosome. At this time, the interchange of material or crossing over from one chromatid to another occur As a result of these changes, the nucleus now contains the haploid number of chromosomes (*i. e.* half of the normal, diploid number) in the form of tetrad (pachytene stage).

The next step in meiosis brings about the separation of the tetrad chromosome into the respective chromatids and as described before involved two division of the cell. These divisions are known as meiotic divisions.

Reductional and equational Meiotic division and the phenomena of crossing over—In the first meiotic division, *i. e.* the first maturation division, if the two chromatids derived from one homologous mate of the tetrad are separated from the two chromatids derived from the other homologous mate, the division is spoken of as reductional or disjunctional. In this case the two associated chromatids of each dyad represent the original chromosome which synapsed at the biginning of meiotic prophase. If however, the separation occurs not in the synaptic plane but in the equational plane, then the two associated chromatids of each dyad come, one from one synaptic mate and one from the other ; such a division is spoken of as an equational division. There appears to be no fixity of procedure relative to the separation of the tetrads, and great variability occurs. However, this may be, one of the two meiotic divisions as far as any particular tetrad is concerned is disjunctional

(reductional) and the other is equational at least in the region of kinetochore. If the first division is reductional than the second division is equational and vice versa. Disjunction in the first maturation division is often referred to as pre-reduction, while that in the second maturation division is called post-reduction.

The foregoing statement about the disjuctional and equational divisions should be considered in the light of the phenomenon of crossing over. In the later process, a gene or groups of genes may pass from one chromatid to the other and vice versa during their association at the four stranded stage. In the region of the centromere fibres and nearby regions, cross over are thought not to occur. Consequently, in the regions of the kinetochore, the statements above regarding disjunctional and equational divisions of the chromosomes appear to be correct. However, the terms disjunctional and equational may mean little in other regions of the chromosomes of the tetrad during the meiotic division in gametogenesis.

Comparison of spermatogenesis and oogenesis—From the above account it is quite clear that both the processes are alike to each other. The following comparison can be made between two processes.

Similarities :—

1. Both the processes start from a primordial germ cell.

2. Both pass through three phases, namely multiplication, growth and maturation.

3. The process of synapsis or pairing of homologous chromosomes takes place in both the processes during the growth phase.

4. In the maturation phase, both have two divisions.

Differences :—

1. The spermatogenesis occurs in the testes while the oogenesis in the ovary cells.

2. The process of spermatogenesis results in the formation of four similar sperm cells from each primary spermatocyte. All these four are functional, while oogenesis results in the formation of only one large cell, from each primary oocyte, the egg cell and three non-functional small cells or polar bodies (polocytes).

3. Mature sperm cells are very small and are also very active. On the other hand mature egg cell, has the large size and is very passive and receptive.

4. All sperms cells are functional and due to much greater number of multiplication, the total number of sperms produced is enormously greater than the number of eggs.

5. Complete process of spermatogenesis finishes in the testes while the oogensis is completed outside the ovary.

GAMETOGENESIS IN PLANTS.

In the evolution of the plant kingdom, there is sinificant shift in dominence from the gametophyte generation to the sporophyte generation. In the lower plants, like algae and bryophytes, the gametophyte stage(during which gametes are formed)is more conspicuous. For example a moss plant is a gametophyte, the sporophyte being small and short lived. The gametophyte tissue is haploid and the gametes are produced by mitosis. Syngamy result in a diploid zygote, which represents the first cell of the sporophyte generation. In ferns this relationship is reversed, the gemetophyte being so small that it commonly escapes notice. In the angiosperms, those plants which bear flowers and seeds, the sporophyte generation is the dominant phase and the gametophyte generation is quite reduced, usually microscope in size.

The cycles are complicated further by variations in the type of sex differenciation. In mosses and ferns the sperms and eggs may be produced by the same gametophyte (**monoecism, homothalism**) or they may occur on different ones (**dioecium, heterothalism**). In the flowering plants, male and female gemetophyte are always distinct and arise respectively from **microspores** and **megaspores**. These spores may be produced by the same sporophyte (**in homophytic plant**) or may be produced by different sporophyte (**in heterophytic plant**).

In a typical angiosperm flower female gametophyte (**megagametophyte**) is produced in the ovary, and male gametophyte (**microgametophyte**) in the anther, by meiosis which than undergo one or more mitotic divisions to give rise to the gametes.

Microsporogenesis and the Male gametophyte— The anther commonly differenciates internally into three regions, *i. e.* an outer **wall** consisting of several layers of cells, a nutritive **tapetum** of one layer, and a central mass of sporogenous cells. The sporogenous cells eventually enlarge as microsporocytes, round up from one another, and lie in the fluid filling the enlarge anther. Each *microsporocyted* or pollen mother cell undergoes meiotic divisions to produce a quartet of microspores, each of which has a single haploid nucleus. In

plants, cytokinesis occurs after division I and again after division II, but in most species it does not take place until after IInd. The microspore is thus the first cell of the male gametophyte generation. Now each microspore nucleus divides by mitosis so that the cell contains two haploid nuclei. Here no cytokinesis (division of the cytoplasm) takes place with the karyokinesis (division of the nucleus). Morphologically the cell increases in size. The walls of the microspore become greatly thickened, the characteristic patterns formed often being useful in the identification of species. In many plants this wall thickening involves the activity of the tapetum. At last it matures into the *pollen grain and its wall consists typically of two distinct layers,* the thickened *exine* and within this an *intine.*

The male gametophyte or pollen grain of angiosperm is structurally very simple. Its development begins with the division of

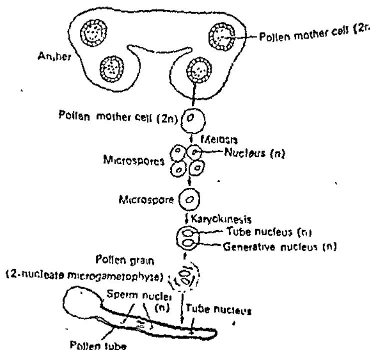


Fig. 95. Microsporogenesis in an angiosperm.

the microspore into a small generative cell and a large tube cell. They may or may not differ in size and shape. The generative cell always lies against the spore wall at one side, or it may be completely enclosed by the cytoplasm of the tube cell. When the pollen grain lands on the stigma of the female reproductive organ, or pistil of a flower,

a pollen tube grows down into the style of the pistil. Thus the tube of the generative nuclei travel into this pollen tube, where another mitotic division occurs.The generative nucleus divides into two sperm nuclei, without accompanying cytokinesis.The tube nucleus functions to form the tube.It does not divide in its whole life span and ultimately degenerates. In some of the cases the division of the generative nucleus so as to form the sperm nuclei or male gametes may occur before the anther opens as in maize.

The time interval for the complete process of sperm nuclei formation varies from one type of plant to another. In some of the monocotyledon plants for example *Tradescantia* (spiderwort), the entire process lasts approximately two weeks. The sperm nuclei are than released into the female gametophyte, where fertilization takes place.

Megasporogenesis and the Female gametophyte—The pistil consists typically of an ovary, a more of less elongated style, and a sticky or hairy stigma upon which the pollens are received. In the ovary there are one or more ovule. Each ovule consists of a central position, the nucellus,surrounded by one or two integuments with an opening, the micropyle. In the nucellus a subepidermal cell, either atonce or after division,enlarges and differenciates as a megasporocyte or megaspore mother cell. The megaspore mother cell first of all enlarges and then undergoes meiosis to produce four megaspores, each having reduced number of chromosomes. In many of the cases these are merely nuclei, with no cell walls. In a typical angiosperm out of these four megaspores, three megaspores degenerate and the fourth one becomes the haploid female gemetophyte. The female gametophyte is considerably larger than the male gametophyte.

Although this process shows many variations in different genera of angiosperms, yet the general structure of the gametophyte is often essentially the same after different modes of development. After this the *nucleus of the female gametophyte undergoes three successive* simple divisions,yielding eight nuclei lying in the common cytoplasm of the embryo sac. The egg apparatus consisting of three nuclei, one egg and two synergids, located at one pole of the embryo sac, *i e*. the micropylar end. At the opposite pole three antipodal cells are located. In the cytoplasm of the sac lie the two polar nuclei ; these are not to be confused with animal polar bodies which are the immediate product of meiosis.

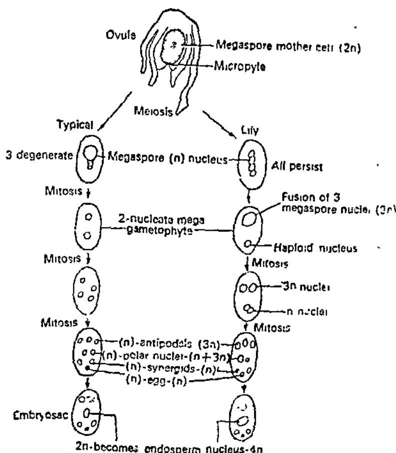


Fig. 96. Megasporogenesis in a typical angiosperms and in Lily.

There are many variations in the megaspore development in angiosperm. Some of the variations are as follows :—

(1) The female gametophyte develops from one of the two cells present after the meiotic divisions I ; two of the nuclei resulting from meiosis thus being involved in the formation of an eight nucleate sac (**Allium-type**) or the four nucleate sac (**Podostemon type**).

(2) In *Oenothera* the single megaspore arises from the four nuclate gametophyte (**Oenothera-type**)·

(3) In the case *Adoxa*, after meiosis there is no cytokinesis and all the four nuclei divide once again to form eight nucleate gametophyte,

(4) The fourth type megagametophyte development has been noted in *Peperomia*. No cytokinesis take place after meiosis and the four nuclei divide twice, giving a sixteen nucleate gametophyte.

(5) In the Lily, Fritillaria-type development takes place. The four haploid products of meiosis persist and take part in the ensuring mitotic division. Out of these four haploid nuclei, one is located at the micropylar end of the ovule and the rest three are located at the antipodal end. The latter fuse to form a *triploid* nucleus. Hence the megagametophyte consists of one haploid nucleus and one triploid nucleus Each of these two nuclei divides by mitosis twice, the result being four haploid nuclei and four triploid nuclei in the embryo sac. The triploid nuclei lie at the antipodal end of the sac while the haploid nuclei lie at the micropylar end. Out of the three micropylar haploid nuclei, two are the synergid and one is the egg. The remaining haploid nucleus and triploid nucleus migrate to the centre of the sac where they fuse to form tetraploid nucleus. When double fertilization occurs,a diploid zygote and a pentaploid endosperm nucleus result.

LIFE CYCLE OF NEUROSPORA

Several micro organisms, including algae, fungi and protozoans have been used in the studies of the mechanism of heredity. We are particularly interested in *Neurospora* because in many ways it offers opportunities for genetic study. The mold and above said microorganisms have atleast two advantages over more highly developed plants and animals One advantage is that they have the quality of rapidly producing the large numbers of offsprings. The second advantage is that some of them are primarily haploid. The significance of this phenomena lies in the fact that an investigator can identify all of the products of a meiotic division. More over, *Neurospora* can be easily kept in pure culture—a great advantage for physiological research. Finally, the nature of its life cycle permits unusually close genetic analysis within a relatively short period, as it predicts the behaviour of chromosomes durng meiosis and the meiotic products in a given instance of sexual reproduction Many of the recent discoveries about the behaviour of the gene and the pattern of inheritance have come from the work on *Neurospora*. For this reason, the life cycle of the *Neurospora*, a pink bread mould has been discussed here.

True gametes are not formed in *Neurospora*. But, the cells that fuse to form the zygote behave just like the gametes in syngamy.

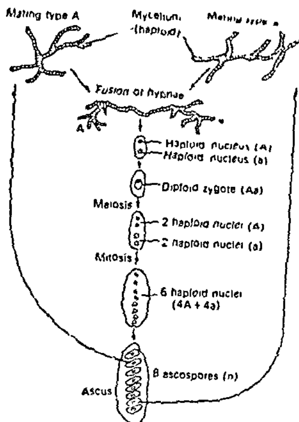


Fig. 97. Life history of Neurospora.

Neurospora has two mating types, which are designated as a and A. Sexual reproduction occurs only when cells of the opposite matting type unite. The gametic cells are derived by mitosis. The fusion *nucleus is the sole diploid stage in this organism. The zygote* quickly undergoes meiosis in the sac-like structure called the ascus so as to form four haploid nuclei. These four haploid products of meiosis than divide mitotically to form eventually eight haploid ascospores. Now, an ascus of *Neurospora* ruptures. Each ascospore is capable of giving rise to a now mycelium. Thus all of the meiotic products persist. By studying the arrangement of the ascospore in the ascus an investigator can determine the behaviour of the chromosome during the division that produce the spores. From the Fig. 97 it is very easy to detect all the meiotic events taken place in the genetic loci of the chromosomes and the consequences of crossing over in the ascospore.

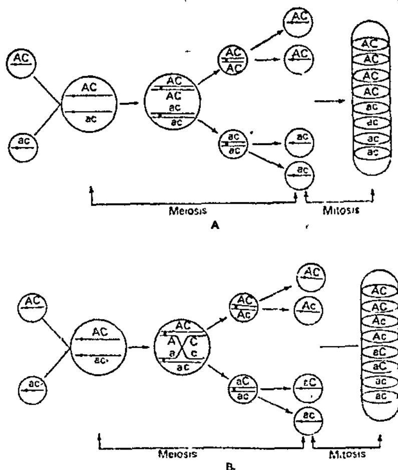


Fig. 98. Genetic pattern in *Neurospora*. A—with no crossing over between the two genes at the time of meiosis. B=with crossing over between the two genes at meiosis A and a=genes for mating type C and c=genes for pink and albino mycelia.

Anomalies during spermatogenesis—There are certain examples where the anomalies occur in the meiotic phenomena of spermatogenesis while the oogenesis remains essentially normal. The few examples are cited below.

In the order Hymenoptera, which includes the wasp, during spermatogenesis all the chromosomes remain unpaired. They are oriented as univalents on the spindle in metaphase I. Since there is no anaphase I, they are all contained in a single nucleus at the end of the I meiotic division. In anaphase II this nucleus u... goes the IInd meiotic division, which is equational. Therefore ca...

of the two (instead of four) products of meiosis contain haploid number of chromosomes. This type of meiosis is under the genetic control.

Two families of the order Diptera, the family Sciaridae and Cecidomyidae exhibit rather stricking meiotic anamolies. In the first meiotic division of seprmatogenesis in *Sclara*,there is no pairing of the chromosomes and consequently no crossing over takes place. The chromosomes of maternal origin are segregated from those paternal origin which separates into a bud that later disintegrate. So at fertilization the male only contributes the maternal chromosomes and never the paternal chromosomes. The second meiotic division is equational for all the chromosomes except the sex-chromosome. So there is no equal distribution of the sex chromosome in forming nuclei. The nuclei without sex-chromosomes degenerate while those containing sex chromosomes become the functional sperms Therefore, the anamolies in meiotic division only produce single functional spermatid instead of the usual four.

The gall midges, show still other peculiarities in their meiotic behaviour during gametogenesis. It has been found that the number of the chromosomes in the somatic cell is quite different from the number found in the germ cells. For example in gall fly, *Miastor* there are 48 chromosomes in a germ cell but only 12 in somatic cell of the female and 6 in a somatic cell of the male. 6 of the chromosomes are referred as S (somatic) chromosomes. The other sets of chromosomes are called E (eliminated) chromosome (since they are eliminated at some time during the developmental cycle. In prophase I of spermatogenesis, the E chromosomes do not pair. One haploid set of S chromosomes (six) plus all the E chromosomes move to one pole,and the other haploid set of S chromosomes moves to opposite pole.In this way the secondary spermatocytes are unequal in size. The larger spermatocytes which contain the E chromosomes undergo degeneration and the smaller ones, having S number of the chromosomes undergo the second meiotic divison which is equational to produce two equal spermatids, each with the haploid number of the chromosomes. In oogenesis six of the S chromosomes (haploid set) are lost in the first meiotic division so that the functional egg contains S chromosomes and E chromosomes. Thus the chromosome number in the *Miastor* egg is 42.

It can be concluded that in its sexual life cycle, gall midge produces only two sperms and each has a typical haploid number of chromosomes. On the other hand, the single egg produced contain many

additional chromosomes, most of them are eliminated during early cleavage. The role of E chromosomes in development is not completely understood.

SUMMARY

Sexual reproduction is a general phenomena among animals.In the multicellular animals, there are two kinds of gametes, which are quite unlike in the vertebrate. The female gamete *i. e.* ovum is inveriably larger than the male gamete, spermatozoon. The process through which the ova and sperms are formed, is known as gemetogenesis ; the process of sperm formation is known as spermatogenesis and the process of ova formation is known as oogenesis.The spermatogenesis occurs in two stages, *i. e.* (1) the formatiom of spermatids and (2) the metamorphosis of spermatids into sperms. The first process, however, is completed in three phase, *i. e.* the phase of maturation, the phase of growth and the phase of maturation. These all three stages are also present in the oogenesis. Most cytological changes occur during the maturation stage in both of the cases. The chromosomes undergo tetrad, and however, crossing over and synapsis formation take place. In metamorphosis of the spermatids, so many changes occur in the shapes and sizes of the spermatids. First of all structural changes occurs such as increase in size : followed by the changes that occur in cytoplasmic inclusions.

In the case of plants this process is somewhat specialized. In evolution there is significant shift in the dominance from the gametophyte generation to the sporophytic generation. In a typical angiosperm female gametophyte (megagametophytes) are produced in the ovary, and male gametophyte (microgametophyte) in the anther, by meiosis which then undergo one or more mitotic division to give rise to the gametes.

There are certain cases, where the anamolies occur in the meiotic phenomena of spermatogenesis while the oogenesis remains essentially normal. The different case may be cited from the class Insecta (orders,—Hymenoptera, Diptera, etc).

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PROTEIN SYNTHESIS

The protein, an agent of biological specificity, is of primary importance to the life of the cell. This constitute the major portions of the dry weight of an actively growing cell. They are not only the main building materials of the cell,but many of them are enzymes and as such they have important functional significance, because all the essential chemical processes going on in the living system are controlled by enzymes. Many genes are known to act through the agency of enzymes. Through their particular composition, organisation and enzymatic activity, protein represents an extremely important group of chemical compounds through which inherited traits are expressed.

Protein structure—Chemically, the proteins are long-chain polymers of aminoacids. It is believed that twenty different aminoacids occur in proteins. All these aminoacids have an asymmetric carbon, which is joined by covalent bonds to four different groups—a **Carboxyle, an amino, hydrogen,** and **R. groups** The R groups are different in different aminoacids, some of them being quite complicated. Amino acids are unusual in that they may act as either an acid or a base because of the presence of the carboxyl and amino groups. Most of the proteins contain between 100 and 1,000 amino acids and have molecular weights from 10,000 to 100,000. These amino acids are linked by a covalent bond called a **peptide bond** between the amino (NH_2) group of one amino acid and the carboxyl (COOH) group of another. In this way truely protein is a polypeptide chain. In each polypeptide chain a free carboxl group always occurs on one end and a free amino to the other (the C-terminal and N-terminal ends).

The complete sequence of aminoacids in a protein is difficult to determine and is known for only in a few proteins. It is this sequence that is determined by the sequence of bases in the DNA. The aminoacid sequence is referred to as primary protein structure,

SIDE-CHAIN (R-GROUP) CHARACTERISTIC	CHEMICAL STRUCTURE	AMINO ACID	
ACIDIC	$O{=}C(NH_2)-CH_2-CH(NH_2)-C(=O)OH$	ASPARTIC	ASP
	$O{=}C(HO)-CH_2-CH_2-CH(NH)-C(=O)OH$	GLUTAMAIC	GLU
BASIC	$NH_2-CH_2-CH_2-CH_2-CH_2-CH(NH_2)-C(=O)OH$	LYSINE	LYS
	$NH_2-C(=NH)-NH-CH_2-CH_2-CH_2-CH(NH)-C(=O)OH$	ARGININE	ARG
	$HC{=}C(-N{=}CH-NH-)-CH_2-CH(NH_2)-C(=O)OH$	HISTIDINE	HIS
SULFUR CONTAINING	$HS-CH_2-CH(NH_2)-C(=O)OH$	CYSTEINE	CYS
	$CH_3-S-CH_2-CH_2-CH(NH_2)-C(=O)OH$	METHIONINE	MET
AMIDES	$O{=}C(NH_2)-CH_2-CH(NH_2)-C(=O)OH$	ASPARAGINE	ASN
	$O{=}C(NH_2)-CH_2-CH_2-CH(NH_2)-C(=O)OH$	GLUTAMINE	GLN
IMINO	CH_2-CH_2, CH_2, $CH-C(=O)OH$, $N-H$ (ring)	PROLINE	PRO

Fig. 99. Different aminoacids found in proteins.

However, the spatial organization of proteins is an important *consideration*. The configuration of the polypeptide back bone refers to the *secondary structure* of protein. Almost all protein exist in a helical configuration called an α-helix. This

SIDE-CHAIN (R-GROUP) CHARACTERISTIC	CHEMICAL STRUCTURE	AMINO ACID	
ALIPHATIC	H — C(H)(NH2)—COOH	GLYCINE	GLY
	CH3 — C(H)(NH2)—COOH	ALANINE	ALA
	(CH3)2CH — C(H)(NH2)—COOH	VALINE	VAL
	(CH3)2CH—CH — C(H)(NH2)—COOH	LEUCINE	LEU
	CH3—CH2—CH(CH3) — C(H)(NH2)—COOH	ISOLEUCINE	ILEU
ALCOHLIC	HO—CH2 — C(H)(NH2)—COOH	SERINE	SER
	CH3—C(H)(OH) — C(H)(NH2)—COOH	THREONINE	THR
AROMATIC	HO—C6H4—CH2 — C(H)(NH2)—COOH	TYROSINE	TYR
	C6H5—CH2 — C(H)(NH2)—COOH	PHENYLALANINE	PHE
	(indole)—C—CH2 — C(H)(NH2)—COOH	TRYPTOPHAN	TRY

Fig. 100. Different aminoacids found in proteins.

helix is stabilized by intramolecular hydrogen bonding and involves the C=O and N—H groups of the peptide chain. The *a*-helix configuration allows the minimum number of hydrogen bonds without distorting bond distances on bond angles. Thus an *a*-helix represents a highly ordered structure. Probably few perfect *a*-helical structures exist, for they would be rigid and rod-shaped. It is more likely that an intermittent ordered structure is characteristic of most proteins. Further, the tertiary structure refers to the topological pattern of the folded chain. The special organization in tertiary structure is stabilized by the side chains of the aminoacids rather than by the peptide backbone. Disulfide bridges are major groups that determine the topology of a folded protein. However, hydrophobic bonds, electrostatic interactions, and hydrogen bonds,

all probably contribute to the side-chain interactions. In this way, the tertiary structure refers to the three dimensional structre of proteins, which is particularly important in the bilogical fuction of certain molecules such as enzymes.

The further important structural consideration in large complex proteins is **quaternary structure.** In this case often two or more polypeptide chains become associated into a complex macromolecule, *i. e.* the biologically functional unit. Certain *enzymes, molecules such as haemoglobin,and the srructural proteins* of cells are dependent for activity on quaternary structure.

Thus a protein is a polymer in which the subunits, (the aminoacids) are joined together in a linear sequence by a simple chemical link. There are about twenty different aminoacid *subunits and a protein chain may contain from less than 50 to* more than 500 of them. After a linear sequence of aminoacids has been arranged most proteins assume a complicated three dimensional configuration, the integrity of which is essential for

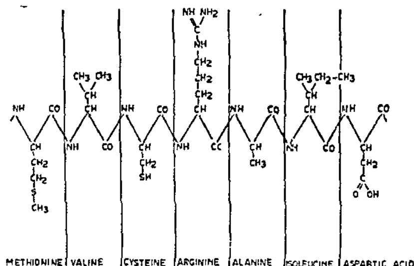


Fig. 101. Typical protein structure.

their biological function. The different properties of the proteins are due to solely different aminoacid sequence. Certain evidences now suggest that twisting and folding of the chain, and the association of chains into large structures, follows automatically from the specification of the amino-acid sequence. **Thus the synthesis of protein, is a process of linking up the unite of aminoacids.**

Site of Protein sythesis—Wilson and Driesh suggested nuclear

origin of protein synthesis. Mazia (1952) proved definitely that the nucleus is necessary for protein synthesis, but not for the production of protein. Recent investigations indicate that the protein synthesis is mostly cytoplasmic in origin. There are mainly two cytoplasmic constituents which contribute to the protein synthesis.

(1) Ribosome.
(2) Mitochondria.

Palade and others in 1956 discovered minute microsomal granules in the cytoplasm, associated with the endoplasmic reticulum, these granules were identified as ribosomes. They may be separated from the endoplasmic reticulum with de-oxy-cholate. They found that they were ribosomes, which contain most of the RNA and preformed protein synthesis. Further experiments reveal direct relationship between ribosomes and protein synthesis. Electron microscope observations showed that these cytoplasmic structures (ribosomes) vary in diameter from 100 to 200A° and contain about 50% protein and 50% ribosomal RNA,

Isolated mitochondria have been found to be the centre for the limited protein synthesis. This fact has been traced out with the help of radio active tracer (C^{14}) even than the ribosomes are the most important centres of protein synthesis.

The Role of Nucleic Acid in Biosythesis of Protein—The nucleic acid plays the main role in transmitting the required genetic informations for protein synthesis There are mainly three kinds of ribonucleic-acids, concerned with protein sythesis. Recent studies indicate that messenger ribonucleic acid (m-RNA) and transfer ribonucleic acid (t-RNA) play the main role in the coding of aminoacids, whereas ribosomal RNA (r-RNA) remain inactive inside the ribosome.

Messenger Ribonucleic acid (m-RNA)—It is an intermediary between the information-carrying DNA and the ribosomes where the proteins are manufactured. The messenger RNA is formed from DNA templates during replication, the base components in m-RNA are complementary to those of DNA. The evidence that DNA is the main source from which RNA is synthesized can be received by using the enzyme deoxyribonuclease, with the result that protein synthesis is stopped. The inhibition of protein synthesis is due to the fact, that m-RNA is not formed due to the absence of DNA as it is absorbed by the enzyme deoxyribonuclease. Hall (1961) gave further evidences that m-RNA is formed only from

DNA. They extracted messenger RNA from the cell and demonstrated that it combines only with DNA, that produce it. For example m-RNA produced by DNA of T_2 bacteriophages upon the infection of *E. coli* would combine only with the T_2 DNA and not with the DNA of *E. coli*.

There are evidences to support the view that m-RNA, carry messages or gene code from the DNA and direct the protein synthesis. Accordingly taking specific aminoacid, *Ameobae* when were cultured in a medium containing radio-active phosphate ions they are taken up by the cell and nucleic acid. Later on these radio-active nucleic acids show the duplicate DNA during cell division, having the same radio active phosphate ion. It is possibie to remove the radio active nucleus from *Amoeba*, with the help of delicate micropipette, This removed nucleus is transplanted to another non radio-active amoeba from which the nucleus has already

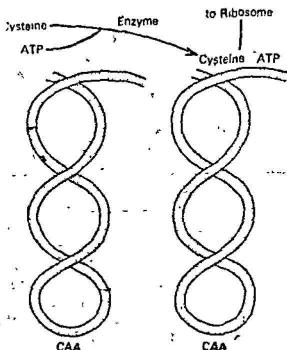


Fig. 102. Diagrams showing the details of the t-RNA activity transporting a particular aminoacid. A transfer RNA molecule with the code CAA at one end picks up the aminoacid cysteine after the aminoacid has united with the energy yielding compound ATP.

been removed. During this period it has been noticed that non radio-active *Amoeba* reveal radio-active cytoplasm and finally this radio activity will be found to be concentrated in the ribosomes. The above experment indicates the role of m-RNA carrying necessary instruction from the DNA and going to the ribosomes for their implantation.

Transfer Ribonucleic acid (t-RNA)—Lipmann and Hoagland (1950) discovered another type of RNA known as t-RNA which is also called soluble or acceptor RNA. A transfer RNA unit transports a single aminoacid from other location in the cell to the m-RNA already placed on the surface of ribosome. An aminoacid to be transported, for protein synthesis, must be activated which is accomplished when the acid becomes coupled with an energy rich molecule of a adenosine triphosphate (ATP) which is already present in the cytoplasm.

The activated aminoacids combine with the unit of t-RNA to form an activated aminoacid complex (AA)-RNA. Each kind of aminoacid combines with a different t-RNA. It means that the number of the RNA always corresponds to the number of the aminoacids present inside the cell. Each t-RNA in this way combines by means of chemical bonding with one and only one aminoacid.

The function of the t-RNA is completed when it delivers the aminoacid ; it is released intact to move in the cytoplasm and pick up another amino-acid and transport it to a ribosome for incorporation into protein. The process go on rapidly in the living cell. In the transfer mechanism specific enzymes are required.

Structurally the t-RNA is a single polynucleotide chain of about 67 nucleotide. In this the RNA molecule is considerable smaller than that of either m-RNA and DNA. t-RNA supperficially appears to be the double helix somewhat similar to the double helix of DNA. The two ends of the t-RNA carry the aminoacids in the form of a code which is usually triplet that means that only three aminoacids are carried by the transfer RNA.

Likewise, the triplet code of the m-RNA is termed as codin whereas the complementary triplet code of t-RNA is called nodoc. Madison and Everett (1967) have suggested the position of the nodoc lies in the middle of the t-RNA molecule.

Ribosomal Ribonuleic Acid (r-RNA)—The bulk of the RNA is called ribosonal RNA (r-RNA), and it is found in the ribosome.

Gentle disruption of cells reveals that the ribosomes can be found to aggregate called polyribosomes or polysomes and it is known that the assembly of aminoacids into proteins occur on the surface of ribosome.

THE GENERAL SCHEME OF PROTEIN SYNTHESIS

Three important key processes may be distinguished in the overall scheme of protein synthesis.

(1) The exact replication of DNA, catalysed perhaps by an enzyme like the DNA polymerase.

2. The transcription of the information contained in DNA into information continued in messenger RNA, presumably by the DNA-dependent RNA polymerase.

3. The translation that take place when the information in messenger RNA is translated into the amino-acid sequence of the newly synthesized peptide chain.

1. **The exact replication** of DNA ensures that all the cells in a multicellular organism will have the same genetic information and also will have the same **potential ability** to synthesize specific protein molecule. Further more the semiconservative mechanism provides for the passing on of the information from generation to generation in all types of organisms. In other words, the exact copying of DNA nucleotide sequence by DNA polymerase provides for the genetic continuity of protein structure.

2. The **transcription process** takes the genetic information from the DNA in the nucleus and place it in the active ribosome fraction in the cytoplasm of the cell, where the actual process of protein synthesis takes place. Further in unicellular organisms specially bacterium, there is probably no problem about the formation of messenger RNA and also the subsequent assembly of the active ribosome, the polysome, from the messenger RNA and inactive ribosomes. In the nucleated metazoan cell, it seems that m-RNA is prepared in the nucleus. However, it is not quite clear, whether the assembly of m-RNA and ribosomes to form the active polysomes take place in the nucleus or in the cytoplasm. If it is found in the nucleus, than the active polysomes would have to move through the holes of the nuclear membrane into the cytoplasm, and indeed it is possible that this is what happens. If active polysomes were formed in the cytoplasm the unprotected m-RNA would have to find its way out of the nucleus into the cytoplasm and these complexes with inactive ribosome to

form the active polyribosome.

The transcription process was thought to be catalysed by the enzyme DNA dependent RNA polymerase.

Watson (1963) pointed out that the translation process in the active ribosome or polysome fraction involves the arrival of amino-acids in the form of activated amino acyl-S-RNA molecules, their assembly in sequence on the m-RNA template in the polysome and the combination of aminoacid to form peptide bonds in the finished peptide chain, followed by the release of s-RNA or t-RNA. The t-RNA is again used and may be viewed as having a catalytic function in the whole process. The process of translation in vivo is an exact one.

In the assembly of peptide, a number of other steps are required. The peptide has to be released from the polysome. It is supposed that a specific enzyme is involved in this process. The peptide chain, specially that of the soluble protein molecule, has to be folded in its correct secondary and tertiary structure. Further more, it is not clear that this process of folding occurs on the polysome as the peptide chain grows, or whether it happens after the peptide chain is released into free solution. From the studies of Epstein, Goldberger and Anfinsen (1963), it is clear that the primary structure of a polypeptide chain has enough information to direct to a considerable extent the folding of the molecule. In this process of folding, the disulphide bonds may be formed from cysteine residues in different part of peptide chain. These disulphide bonds would help to stabilize the secondary and tertiary structure of the folded peptide chain. However, the two processes, the inherent perference of a given aminoacid sequence for a certain tertiary structure, and the formation of the stabilizing disulphide bridge, finally results in a fully folded protein subunit.

enzyme. In the same way, in protein that contain in addition to the polypeptide chain also carbohydrate residues or lipid residues, these are generally attached themselves covalently, probably under the action of special enzymes. In general, however, we can speculate that this attachment of any group occurs at the stage of protein synthesis when the peptide chain is already folded in its correct secondary and tertiary structure.

Many proteins in the organisms are composed of more than one subunits, *i. e.* more than one peptide chains. The assembly

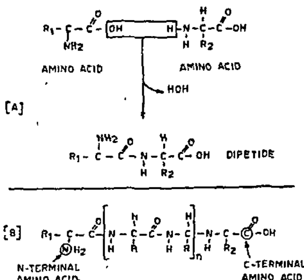


Fig. 103. The formation of peptide chain.

of subunits into the final protein is the last step in the synthesis of such protein. It may or may not be accompained by the formation of disulphide bonds between subunits. In the case of haemoglobin, the subunits are held together by electrostatic interaction, some hydrogen bonding and specific surface fit between the subunits ; but no special enezyme is required to carry out this assembly (Moore, *1965), and the submits come together in free solution. Although* we know considerably about the various stages in the synthesis of a soluble protein, but, however, we know very little about the final stages of protein synthesis of structural and insoluble proteins. Moore (1965) pointed out that the assembly of the primary amino-acid sequence of structural protein is the same as for soluable protein but the mechanisms by which these are bound with carbohydrate or lipid components and assembly into such structures (as cell membrane) is almost completely unknown.

MECHANISM OF PROTEIN SYNTHESIS

First of all Lipmann presented the idea for the protein synthesis suggesting that different aminoacids must be brought into a high

energy" state. He further hypothesized that this could be done only through the mediation of ATP. Hoagland later discovered the aminoacid activating enzymes. ATP, however react with an individual aminoacid, in the presence of this enzyme, to split off inorganic pyro-phosphate and form an aminoacyle adenylate. The compound remains tightly bound to the enzyme. There are specific enzymes catalyzing the reaction for each individual aminoacid. The complete picture of the entire mechanism occurs in the following steps.

1. The Activation and charging reaction.
2. The Transfer Reaction.

The Activation and charging reaction—The initial stage in proteins synthesis consists of activation of different aminoacids by the reaction involving the energy-stage molecule, adenosine triphosphate (ATP). Most of the aminoacids prior to this remain in the inactive stage in the cytoplasm. In this reaction, the carboxyl (COOH) group of the aminoacid react with ATP forming aminoacyl adenylate and releasing pyrophosphate.

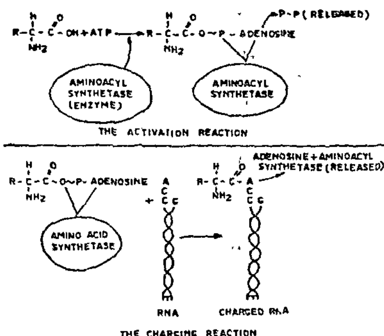


Fig. 104. Showing the activation and charging reaction.

A large number of the enzymes catalyze the activation, but each is specific for a particular aminoacid. This means at least

twenty enzymes must function at this step. Magnesium ion and as well as ATP is inovled in the reaction.

Attachment of activated aminoacid with t-RNA—Enzyme-bound aminoacyl adenylate reacts immediately with transfer RNA and thus form an aminoacyle t-RNA product. The same enzyme that is envolved in this reaction also functions to transfer the amino-acid to t-RNA. These enzymes are then called aminoacyl-tRNA synthetases. They are unique catalysts, as they not only activate the aminoacid but are also capable of recognizing a particular t-RNA molecule. It is however, possible that structural changes in a particular synthetase might result in alteration of the coding relationships in that organism. Aminoacyl-t-RNA synthetases are known to differ in different organism. However, the level of mistake is quite low probably because no more than one incorrect amino acid in several thousand transferred. As pointed out earlier t-RNA are small molecule consisting of only seventy-five or eighty bases. The aminoacid is transferred to the adenine (A) that is present on one end of all t-RNA molecules. The important match that must be met,involves a unique triplet of bases in t-RNA usually called the anticodon. The anticodon is complementary with a triplet (the codon) present in m-RNA. If, however, a wrong aminoacid becomes attached to a particular t-RNA, that aminoacid will be inserted into the growing peptide chain.

2. **The transfer reaction**—This is the most important step in protein synthesis. This complete reaction involves aminoacyl t-RNA, ribosome, and m-RNA—resulting in peptide-bond formation. Two enzymes are required, as well as guanosine triphosphate (GTP) and several inorganic ions. Not only these three important components interact but they do so in a dyanamic way. First m-RNA moves towards the ribosome :.then t-RNA molecule moves in, become positioned and enzyme interaction occurs.

✓The first step in transfer reaction is the **binding reation** which occurs between aminoacyl t-RNA and the m-RNA ribosome complex. This reaction appears to be a nonenzymatic, and both charged and uncharged t-RNAs are bound. The binding is very specific. It is however, employed for stuides of the genetic code.

Further the attachment of the ribsome to the messenger is also quite specific and is always occur in a single site on the 5′-hydroxyl end of the m-RNA. This corresponds to the position of

the triplet coding for the N-terminal aminoacid in the completed protein. Perhaps, the attachment of ribosome to m-RNA may be rate limiting in protein synthesis. It has been studied by scientists that certain antibiotics such as tetracyclines may inhibit protein synthesis by interfering with the binding reaction of aminoacyl-t-RNA. However, they do not appear to interfere with m-RNA-ribosome attachment.

Two enzyme fractions function in peptide synthesis one of which involves GTP. Only one t-RNA can bound with each ribosome in the absence of enzyme fraction. However, when the enzyme is present, a second t-RNA can be bound to the same ribosome. This behaviour suggests that the enzyme function modify the ribosome structure thus producing the new binding site and moving the ribosome to the next codon on m-RNA. Meanwhile, the peptidyl-RNA already attached to the ribosome to prepared acelate, the new aminoacyl t-RNA, *i. e.* bound to a new site. Further the peptide-bond synthsis involves condensation of two aminoacyl-tRNA to yield peptidyl-RNA, and free RNA, which remain bound to the ribosome.

Further studies on the process, indicate that the antibiotic puromycin appears to replace aminoacyl t-RNA and to form a false peptide bond, thus resulting a chain termination. Thus, it stops protein synthesis and becomes the C-terminal of the released peptide chain. This release occurs because puromycin will not bind to the ribosome.

Not only this but still a third ribosome site has been proposed for the uncharged RNA that has just been released by peptide-bond formation. In short, these three sites would be the deconding, condensing and exit sites. It is unknown whether a particular site can serve any function through enzymatic action.

Still another antibiotic, chloramphenical, has long been known to be an inhibitor of protein synthesis. It appears to funtion in the transfer reaction at the step of peptide-bond synthesis. Attachment of m-RNA to ribosomes seems to occur normally, but synthesis seldom proceeds past the di or tripeptide stage. The inhibitor is probably bound to a specific site on the ribosome. The another important inhibitor of protein synthesis, cycloheximide is, not effective in bacteria but seems to function in mammals and fungi in a similar way as does chloramphenicol.

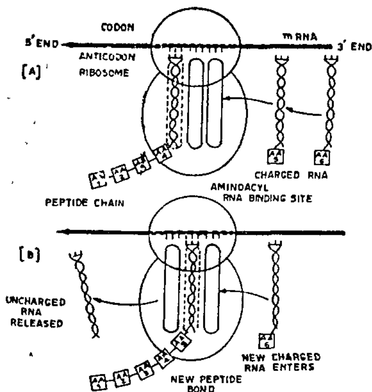


Fig. 105. Diagram showing the transfer reaction.

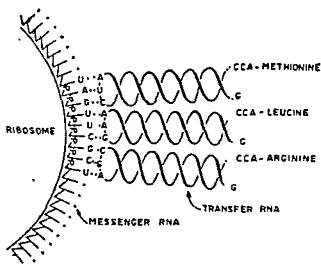


Fig. 106. Assembly of aminoacyl-s-RNA in a template.

POLYSOME—*Witson* and *Tissieres* gave the idea about the number of ribosomes which are attached to a single m-RNA molecule. *Messenger RNA derived by transcription of DNA rapidly* become associated with ribosomes. Presumably, the ribosome can only attach at the proper *position for translation of* a peptide form its N-terminal beginning end. As protein synthesis proceeds, a number of ribosomes become associated with a single m-RNA; these structures are called **polysomes.** They have been however observed by electron microscopy as well as recognized indirectly through density gradient fractionation of the cell. Warner and **Rich** (1964) have discovered that in mammalian reticulocyte cells there are seven or eight ribosomes attached linearly along the m-RNA molecule forming polysome. These polysomes complexes are much more active in protein synthesis than single ribosome.

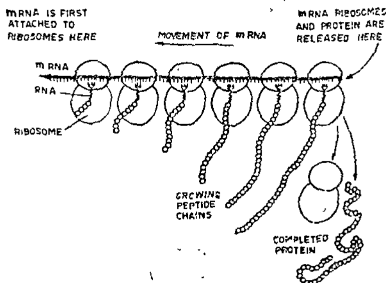


Fig. 107. Diagrammatic representation of a polysome and *the attachment of aminoacids on m-RNA.*

Studies on lebeling techniques have proved to be useful to identify m-RNA as a component of polysomes. *Further indication* that protein synthesis occurs on polysome structures has been obtained with *aminoacid incorporation experiments. However,* in the case of a polycistronic or multigenic message, it is not clear whether ribosomes can be attached at various places along the message or only at the begining of the long chain. *In the case of* higher organisms, the ribosomes are found to be attached with

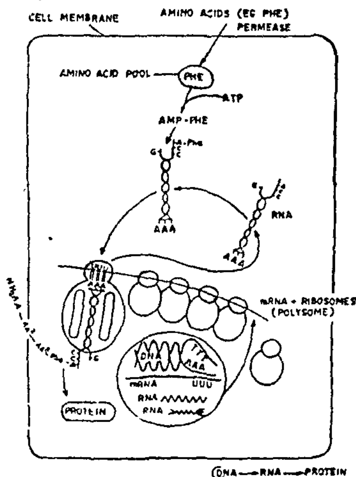


Fig. 108. The cellular view of protein synthesis.

membranes,—so it is probable that movement of m-RNA occurs along ribosomes rather than the reverse. However, in any case m-RNA binds to the smaller 30-S subunit of ribosomes and t-RNA binds to the larger 30-S subunits. Very recently, cytoplasmic factors have been recognized as being involved in chain initiation.

PROTEIN SYNTHESIS IN NON NUCLEATED CELLS

If the nucleus from any cell is removed,the local source of messenger RNA to the ribosomes is no doubt cut off,but the ribosomes can continue to synthesize proteins for a short time because of the existing supply of m-RNA which was already in the cytoplasm. In time, however, the synthesis stops since messenger RNA is destroyed after its use of protein synthesis and eventually the cell dies. The human red blood cells are nonnucleated as they normally exist in the

blood stream. This is the question how they (red blood cell) can continue the protein synthesis and other activities without nucleus, chromosomes and genes. The red blood cells are produced in the red bone marrow and they contain nuclei in their premature stage. However, sufficient messages are sent to the ribosomes and sufficient protein is produced to keep the cell alive and active even after the nucleus has been expelled.The red cells do not continue to grow and synthesize new protein, however after they are released into the blood stream, and their life span is limited to about 120 days.

THE GENETIC CODE

DNA is known to be a very long molecule which resembles a ladder that has been twisted into a helix. The side of the ladder are formed by alternating units of the deoxyribose and phosphate groups ; the rungs, which join two deoxyribose units are composed of pairs of bases, either adenine paired with thymine or guanine paired with cytosine. The molecule of DNA unwind and splits into two strands. Each strand can either replicate itself, or however, direct the manufacture of the strand of messenger RNA. If RNA is formed, the rungs of the ladder when one side is DNA and other side is RNA, than the guanine paire with cytosine and adenine with uracil as follows.

DNA	RNA
T	A
G	C
G	C
A	U
G	C
T	A
T	A
T	A
A	U
A	U
T	A
G	C
C	G
C	G
A	U

Fig. 109. Formation of RNA strand by DNA. During RNA formation adenine of the DNA molecule will link with Uracil instead of Thymine,

Thus formed messenger RNA moves from the nucleus to the cytoplasm, where with the help of transfer RNA, it directs the formation of protein by determing the exact sequence of amino-acids in the protein chain. But the question is, where does the information lie. If we look toward the DNA chain, deoxyribophosphate backbone seems to be of no help because it is a repeating structure. The only hope lies in the four bases occuring a large number of time and having the immense number of possibilities of sequentional arrangement. The four bases of DNA molecule keep in a coded form of message for the structure of protein molecule. This is what we mean by "genetic code". But again there is a problem :-how can these four bases control the sequence of different twenty aminoacids ?

A moment of reflection and the use of simple calculation suggest the answar of the problem. If, each base has the responsibility of attracting a specific aminoacid, *i. e.* 1 : 1 relationship, peptides containing only four aminoacids will result But there are

TABLE 7—POSSIBLE CODON FOR m-RNA

Singlet Code (words)	Doublet code (16 words)				Triplet Code (64 words)			
					AAA	AAG	AAC	AAU
					AGA	AGG	AGC	AGU
					ACA	ACG	ACC	ACU
					AUA	AUG	AUC	AUU
					GAA	GAG	GAC	GAU
A					GGA	GGG	GGC	GGU
G	AA	AG	AC	AU	GCA	GCG	GCC	GCU
C	GA	GG	GC	GU	GUA	GUG	GUC	GUU
U	CA	CG	CC	CU	CAA	CAG	CAC	CAU
	UA	UG	UG	UU	CGA	CGG	CGC	CGU
					CCA	CCG	CCC	CCU
					CUA	CUG	CUC	CUU
					UAA	UAG	UAG	UAU
					UGA	UGG	UGC	UGU
					UCA	UCG	UCC	UCU
					UUA	UUG	UUC	UUU

Showing the code letter combinations. If single base pro-

code employs triplet codon.

twenty aminoacids and there are polypeptides with all twenty in a bewildering variety of combinations. If, however, a relation is assumed to be combination of two bases for each amino acid, sixteen combinations are possible, but *i. e.* still too few. If the units each contain 3 bases, 64 combinations may be formed which are more than enough. The table given on the previous page provides all the possible combinations using 1, 2 and three bases units.

The word "Code" has several meanings. Out of them one of which is "a system of signals for communicating". A pertinent example is the Morse code. In this chapter, we are dealing with the code, too for messenger RNA has a means for communicating message that direct the combination of aminoacids in a specific sequence. Geoge Gamow first proposed the coding scheme in 1954. Also as a part of our language is the term codon which refer to the group of bases that codes one aminoacid. Gamow, however, concluded that each codon contains three bases and that adjacent codons overlap. If this were true then :—

(1) Only certain aminoacids can follow others, and

(2) A change in a single base leads to change in three adjacent aminoacids. The evidence, however, shows no restriction on aminoacids sequence, and single aminoacid can be changed. For this, a non-overlapping code was proposed.

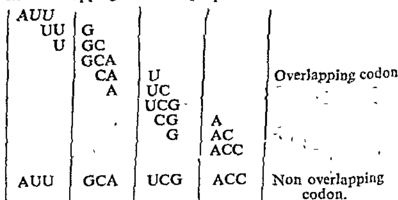


Fig. 110. Genetic code first of all proposed by Geoge Gamow as three bases codon that overlapped. But now evidences suggest that the codons do not overlap.

The evidences for the nonoverlapping nature of the code comes from the analysis of certainamino acid seque[illegible] proteins such as the code protein of TMV and the t[illegible]nthetase of *E coli.* For example, the [illegible] of TM[illegible] amino-

acid sequence of the cod protein are, for the most part, changes in only a single aminoacid. Whenever, two aminoacids are changed (although very rare) they are not in adjacent position. If, however, the code were an overlapping triplet code, a change in one of the nucleotide of a codon could change as many as three adjacent aminoacids in the protein. As well as similar observations have been made with respect to *E. coli* tryptophan synthetase.

Although, at present, the triplet, *i. e.* three base codon concept was generally accepted by all, but the fact that there were sixty four possible triplets and only twenty aminoacids has raised the problem before the investigators. Since there are only twenty amino-acids, twenty triplets would,therefore, seems, sufficient to code the different aminoacids.For what the rest fourty four triplets are there?

CAT	CAT	CAT	CAT	CAT	CAT	CAT
CAT	CAG	TCA	TCA	TCA	TCA	TCA
	↑					
CAT	CAG	TCG	ATC	ATC	ATC	ATC
	↑	↑				
CAT	CAG	TCG	ATG	CAT	CAT	CAT
	↑	↑	↑			

Fig 111. Proof that codons contain three bases of multiple of threes.

The suggestion was made by Crick and others that perhaps two or more triplets stand for each aminoacid, or that some triplets have nothing to do with aminoacid but serves some other purposes. They could say, in genetic code, "begin here" or "end here". What-ever may be their purpose, triplets that do not represent an amino-acid are now referred as nonsense tripletes—a not altogether appro-priate term.

Still there was no evidence that a codon contains three bases rather than four or even more. Crick, however, has now complied compelling evidences for the triplet concept. But using the method

which permits removing or adding bases to genes in T_4, he found that the addition of one or two bases disrupts the entire sequence, but the addition of the third base in the sequence brings the original message back in phase as shown in Fig 112. This argues strongly for the triplet concept but it does not rule out the possibility that codon may be made up of multiple of 3.

	U		C		A		G		
	UUU		UCU		UAU		UGU		U
U	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA		UAA	OCHRE	UGA	*	A
	UUG		UCG		UAG	AMBER	UGG	Tryp	G
	CUU		CCU		CAU	His	CGU		U
C	CUC	Leu	CCC	Pro	CAC		CGC	Arg	C
	CUA		CCA		CAA	GluN	CGA		A
	CUG		CCG		CAG		CGG		G
	AUU		ACU		AAU	AspN	AGU	Ser	U
A	AUC	Ileu	ACC	Thr	AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
	GUU		GCU		GAU	Asp	GGU		U
G	GUC	Val	GCC	Ala	GAC		GGC	Gly	C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Fig. 112. The genetic code and 64 codon along with aminoacids which they represent in the code.

At last returning to the unravelling of the genetic code, Crick and others working with T_4 have presented evidences which strongly suggest that the message on messenger RNA begin probably at one end of the gene and read three bases at a time. *In some* way, yet not clear, the synthesis of the protein begins at the begining of a particular gene and end at the end of gene ; contiguons genes on th RNA molecule have no influence on that particular *protein*. As already indicated, the nonsense triplet may isolate the genes *from* each other.

THE GENETIC CODE DICTIONARY AND CELL FREE SYSTEM OF PROTEIN SYNTHESIS

Progress in this field has been so rapid and amazing *that not* only has the genetic code be broken but now *it is possible to make* a start, at least, in the compilation of a genetic code dictionary.

This is because of the work of many investigator. But particularly Authur Kornberg who first of all synthesized DNA, Severo Ochoa who succeeded making RNA chain with one base rather than the usual four, Beadle, Benzer, Watson and Crick, already mentioned, and particularly two young biochemists Marshall Nirenberg and Heinrich Matthaei.

In 1961, Nirenberg and Matthaei at United States National Institute of health made the brillient discovery that synthetic polyribonucleotides can stimulate the incorporation of aminoacid into peptide like material in the cell free system derived from *Escherichia coli.* The cells grow rapidly, are than collected and broken open be grinding. This releases the cell sap which contains DNA, messenger RNA, ribosome, and other ingredients necessary for the incorporation of aminoacids into protein. A significant feature of the synthetic polyribonucleotides is that they can be made with a known base composition. For example it is possible to prepare polyurodylic acid (poly U), polyadenylic acid (Poly A) and polycytidylic acid (poly C) and so forth. Further they add one of these synthetic nucleotide to a mixture of cell-free extract and the twenty aminoacids. In each mixture one of the amino acids contain radioactive carbon 14. The cell-free extract is first treated with deoxyribonuclease, an enzyme that specially destroys DNA. Without DNA, the cell RNA is soon depleted and protein synthesis stops. The addition of RNA restarts protein synthesis. This fact, however provided Nirenberg and Matthaei a marvelous tool for further researches. They infact replaced the cell messsenger RNA with their synthetic RNA which contained but one base. Protein synthesis took place, the protein was extracted and analyzed. It has been observed that with poly U as the messenger RNA in a protein synthesizing system, the polypeptide so formed contains only one aminoacid, *i. e.* phenylalanine. This simply shows that uracil triplets can code for phenylalanine. With poly A as m-RNA, a polypeptide is formed containing only the aminoacid lysine, and when poly C is used, the aminoacid proline is formed. Thus it is clear by their experiments that adenine triplets can code for lycine and cytosine triplets can code for proline. Poly G does not seem to control any one of the aminoacids.

Further Nirenberg and Matthaei also used synthetic RNA containing bases in various combinations or mixed polymers. For example Polyadenine—cytosine (Poly A-C), when it is used as

m-RNA, six different aminoacids are incorporated into the polypeptide *i. e.* asparagine, glutamine, histidine, proline, threonine and lysine.

TABLE 8—THE AMINOACID CODE[1]

Aminoacid	Nucleotide triplet or RNA Code word[2].
Alanine	CUG CAG CCG
Argenine	GUC GAA GCC CGC
Asparagine	UAA CUA CAA ACA
Aspartic acid	GUA GCA ACA
Cysteine	GUU UUG UGG
Glutamic acid	AAG AUG AGU AGA
Glutamine	AGG AAG
Glycine	GUG GAG GCG UGG
Histidine	AUC ACC
Isoleucine	UUA AAU
Leucine	UAU UUC UGU GUU
Lysin	AUA AAA AAC AGA
Methionine	UGA
Phenylalanine	UUU UUG
Proline	CUC CCC CAC CCU
Serine	CUU CCU ACG UCG
Threonine	UCA ACA CGC CAA
Tryptophan	UGG
Tyrosine	AUU UAU
Valine	UUG UGU

1. From Wahba *et al*, Proc. Natl, Acad. Sci. U. S. 49 (1) 116 (1963)

2. A. adenylic acid ; U, uridylic acid ; G. guanalic acid ; C, cytidylic acid.

If, however, a mixed polymer is synthesized with different ratio of A and C, than it has been noted that when the amount of A is more than C, the ratio of asparagine to histodine in the polypeptide increases. Finally they synthesized a polymer in which there is a regular base sequence. For example a regular Co-polymer of C and U (CU CU CU CU CU CU......). When the C-U co-polymer is used as the m-RNA, two different aminoacids leucine and serine, are incorporated into the polypeptide. The table 8 lists the code words that correspond to each of the twenty aminoacids. The genetic code dictionary assumes that all the codons are triplet.

Now we should think about the assignment of specific codon for the incorporation of specific aminoacids into protein. It should keep in mind that a given codon in DNA (for example CAT) is transcribed to *m*-RNA as the complimentary GUA. It is clear that G and C are complimentary bases and U replace T in RNA. Thus, A and U are complementary. It is an usual practice to refer the codon in the terms of the RNA bases instead of DNA bases of which the ultimate codon are comprised.

Trinucleotide Binding Studies

Later Nirenberg devised a more elegant and useful technique for determining the genetic coding system. The method is known as "binding technique". He pointed out that the addition of trinucleotides (3-RNA bases) to the cell free system causes the binding of a specific charged t-RNA to the ribosomes. The various trinucleotides were prepared and binding experiments were carried out. Each trinucleotide was tested against all twenty t-RNA's, one of which was charged with a radioactive aminoacid. Ribosome can however, be prepared from free t-RNA by filteration on a membrane filter so that the bound radioactivity can be counted and matched with the trinucleotide in question. For example the 5'CAC3' trinucleotide promotes the binding of histidine-charged t-RNA to ribosome. In this way most of the sixty four possible trinucleotides have been synthesized as given in table by the help of the m-RNA in the form of the synthetic polyribonucleotides and also by taking advantage of the fact that S-RNA will bind the ribosome.

However, the table of the previous page represents the combined coding data from polypeptide synthesis or t-RNA binding experiment. It can be seen that the code is highly degenerate. In the language of coding, a code with multiple words for each object coded is said to be degenerate. Secondly the codon are ambiguous, *i. e.* specificity of

a codon for one aminoacid is not absolute.

Degeneracy in the codon probably occurs because a variety of t-RNA molecules with differing anticodon triplets are charged with the same aminoacid (different words may mean the same amino-acid.) Secondly the ambiguity is probably due to different amino-acids being attached to a particular t-RNA with a *simple anti-*codon triplets one word may mean different aminoacids. Certain (antibiotics also result in the attachment of the wrong amino acid to a particular t-RNA. Although it has been possible to assign codons for the twenty aminoacids. But as there are certain ambiguities, therefore the code is not perfeet.

Although the binding of the first t-RNA appears to be non-enzymatic, but it may develop that enzyme play a *specifying* role in vivo. It has been noted that metabolic or chemically related aminoacids have similar codons. **Jucks** has suggested that there are fifteen doublet codes and, as new aminoacids evolved, the third base did also, resulting a triplet cod. Actually more information is required than is present in a doublet code (sixteen combinations) to accomodate all aminoacids.

With this, there are certain cases of codons that do not appear to code for any aminoacid. Such nonsense codon *probably play* a role in starting and stopping the reading of a genetic message.

Although progress has been rapid and the results *remarkable*, many important questions remain to be answered. No doubt *proteins* are the most important single compound in living things; but understanding the way in which the specific proteins are manufactured in cell is a long way from understanding, why certain cells become blood vessels and other make up the brain. To be very sure, even with only twenty aminoacids the number of possible types of proteins is astronomical. In short, the possibilities are understood but not the mechanism.

Specific protein sythesis—In bacteria however it is *easy to* produce certain new proteins. For this synthesis new enzymes can be induced by the addition of the substrate in the culture *medium*. The synthesis of new specific proteins start immediately upon addition of substrate. This synthesis also ceases very quickly after the bacterial cell is washed free of the substrate. When bacteria are infected by bacterial viruses or bacteriophage, the infected cells begin very quickly to form new enzymes necessary for the new synthesis of specific vital constituents.

Genetical implications—However, the codes have a great genetic

implications in the human population. However, several type of anemia appear in human population. Sickle cell anemia, which is found primarily in negroes, is characterised by red blood cells that assume abnormal shape which however interferes with normal circulation. The organism with this affliction are weak and poorly developed and have a varying degree of tissue demage. The analysis of various haemoglobin, from the anemia individuals, indicated that an alteration in aminoacid composition of one portion of the haemoglobin molecule is responsible for the condition. In sickle cell anemia haemoglobin, the aminoacid, valine has replaced the aminoacid glutamic acid of normal haemoglobin. This substitution can be interpreted as a single base change in the triplet genetic code of messenger RNA, the adenine member of the triplet has been replaced by *uracil*, so that the code changes from AUG to UUG. Similar abnormalities in the haemoglobin *is* also brought about by the changes in the substitution of aminoacid.

SUMMARY

The protein, an agent of biological specificity, is of primary importance to the life of the cell. This constitute the major portion of the body cell. Proteins are long chain polymers of aminoacid and large number of different aminoacids occur in protein. These aminoacids have an asymetrical carbon which is joined by covalent bonds to four different groups—a carboxyl, an amino, an hydrogen and a R group. The complete sequence of aminoacids in a protein is difficult to determine and is known for only a few proteins. It is the sequence which is determined by the properties of the proteins are due solely to different aminoacid sequence. It is now supposed that disulphide bridges are major group that determine the topology of a folded protein. However, hydrophobic bonds, electrostatic interaction, and hydrogen bonds all probably contribute the side-chain interactions.

The over all scheme of protein synthesis can be classified into three important structure. First is the exact replication of DNA catalysed. The second process is the transcription of the information contained in DNA information contained in m-RNA.The third process is the translation that take place when the information in messenger RNA is translated in the aminoacid sequence

of the newly synthesis peptide chain. The activation of aminoacids is brought about by ATP. The large number of the enzymes catalize the activation, but each is specific for a particular aminoacid. The polysome units have been the very important units for protein synthesis. The number of the ribosome particles which form the polysome, with m-RNA, varies in number in different cell. In mammalian reticulocyte cells there are seven or eight ribosomes arranged linearly along the m-RNA.

The nonnucleated cells as red blood corpuscles in man, manufacture the protein with the preexisting information gathered in the premature stages when they contain nucleus. The genetic code involves a series of triplet, called codons, which are specific for each aminoacid. Each transfer RNA has one of the possible 64 codons. The genetic code dictionary lists the 64 codons and their *aminoacids.*

It is now possible to synthesize the protein with the all important component which are need in *vitro.* Nirenberg and Matthaei (1961) has successfully synthesized the protein from few aminoacids.

Although much progress has been made in the field, but understanding the ways in which the specific proteins are manufactured in cell, is a long way from understanding.

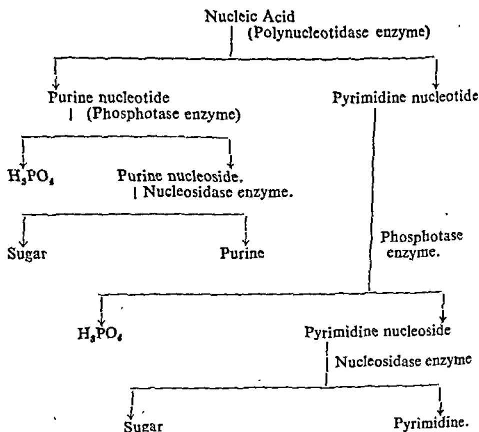
[illegible]

[illegible]

[illegible]

[illegible]

specific enzyme. The nucleic acid is first hydrolyzed by an enzyme callad polynucleotidase to form nucleotide. The nucleotides are hydrolysed by an enzyme called phosphotase to form nucleosides. In this reaction phosphoric acid is removed from each nucleotide. Finally an enzyme called nucleosidase splits the nucleotide into sugar and base. The base may be of two types. The first is the purine base while the second is the pyrimidine base.



The followings are the important constituent which are formed after hydrolysis.

Pyrimidine bases—The pyrimidine bases are all derivatives of the parent compound pyrimidine, and the derivative found in the nucleic acids are *cytosine*, found in both the nucleic acid, uracil found in RNA, and *thymine* and 5-methylcytosine found in DNA. A fifth pyrimidine base 5-hydroxymethylcytosine, *replace cytosine*

in certain strains of coliphage. The formulae shown here are conventional but it must be remembered that keto-enol isomerism is a general property of purine and pyrimidine derivatives.

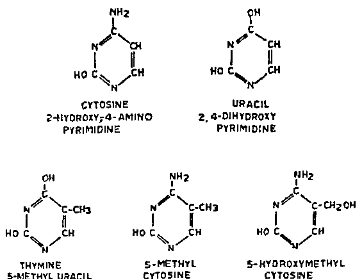


Fig. 113. Structural formulae of pyrimidine.

Purine bases—Both types of nucleic acids contain the same purine bases, adenine and guanine. They are derivatives of the parent compound purine which is formed by the fusion of a pyrimidine ring and an iminazole ring. Adenine and guanine have the following structure.

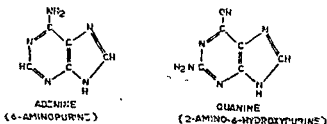


Fig. 114. Diagram showing purine structure.

Other naturally occuring purine derivatives include hypoxanthine, xanthine, and uric acid. Certain minor bases are also present in some nucleic acids in small amounts. For example, the so called "soluble" or "transfer" RNA may contain such

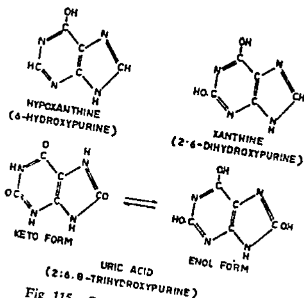


Fig 115. Structure of different purine.

methylated bases as 2-methyladenine, 6-methylaminopurine, 6-dimethylaminopurine, 1-methylguanine, 6 hydroxy–2-methylamino purine, 5 methylcytosine and even thymine. These unusual bases comprise less than 5 percent of the total base content of the s-RNA and vary in relative amounts from species to species.

Pentose Sugar—It has long been recognised that the nucleic acid originally prepared from yeast contained a pentose sugar which was identified as ribose by Levene (1909). Subsequently Gulland (1943) proved without doubt that the pentose in the yeast RNA is D-ribose The sugar component in liver RNA has been proved to be ribose by identification as the *P*- bromophylhydrazone. The pentose sugar from several strains of tobacco mosaic virus (TMV) have been identified as ribose by conversion to the di-n-propyl mercaptals. Some RNA's contain very small amounts of 2' (3')-O-methylribose,

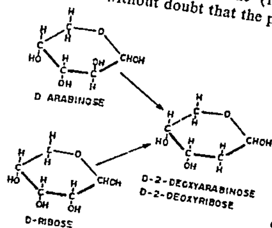


Fig. 116. Formulae of deoxyribose sugar.

Levene and Mori (1929) isolated the sugar from the guanine nucleoside of this nucleic acid and showed that it was a deoxypentose. Only two-deoxypentose sugar can exist, deoxyribose or ribodeose (arabinodeose) derived from ribose and arabinose, and

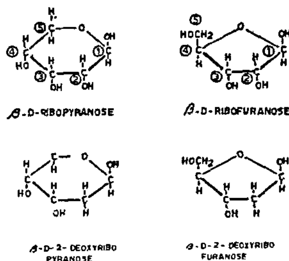


Fig. 117. Showing the structure of different pentose sugars.

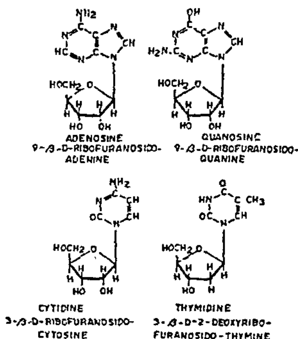


Fig. 118. Showing the structure of different nucleosides.

deoxyxylose or xylodeose (lyxodeose) derived from xylose and lyxose. Since the sugars in the DNA's from several mammalian tissues, from various microorganisms and from fish sperm, have been identified as deoxyribose sugar. Further glucose occurs glycosidically linked to hydroxymethyl cytosine in the DNA from certain strains of bacteriophage.

Nucleoside—A purine or pyramidine base may be condensed with a pentose or a deoxypentose sugar to form a *nucleoside*. Thus adenine condenses with ribose to form the nucleoside *adenosine*, guanine forms *guanosine*, cytosine forms cytidine and uracil forms *uridine*. The ribonucleoside from the hypoxanthine is named inosine. The structure of the four important type of nucleosides are given in Fig. 118. These nucleosides are present in RNA's. The corresponding nucleosides obtained from DNA are formulated and commonly referred to respectively as *deoxyguanosine, deoxyadenosine, deoxycytidine* and *thymidine*.

Nucleotides—The nucleotides are all the phosphoric esters of the nucleosides. Those derived from ribose nucleosides are usually referred to as ribonucleotides and those from deoxyribose nucleosides as deoxyribonucleosides. Previously these terms have commonly been abbreviated to riboside, ribotide, deoxyriboside and deoxyribotide. *Because the ribose nucleosides have free hydroxyl groups* on the sugar ring, three possible nucleoside monophosphates can be formed. Thus adenosine can give rise to three monophosphates (adenylic acids), adenosine 5'-phosphate, adenosine 3'-phosphate and adenosine 2-phosphate. The first of these was originally discovered in the free state in muscles and referred to as muscle adenylic acid, while the second was originally obtained from alkaline hydrolysates of yeast-RNA and used to be called yeast adenylic acid. In the same way guanosine, cytidine and uridine can give rise to three guanosine monophosphates (guanylic acids), three cytidine monophosphates (cytidytic acid), and three uridine monophosphates (uridytic acids) respectively. The structure of nucleotide or nucleoside monophosphates may be represented as follow.

The nucleoside 5'-phosphates may be further phosphorylated at position 5' to yield di and triphosphate. Thus adenosine 5'-phosphate (AMP) yield adenosine diphosphate (ADP) and, adenosine triphosphate (ATP). The structures of these compounds have been confirmed by periodate oxidation and by synthesis.

Further, in the same way other nucleoside 5'-phosphates yield

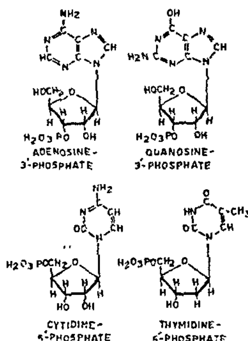


Fig. 119. Showing the structure of different nucleotids.

such di and triphosphates as GDP, CDP, UDP, GTP, CTP and UTP. The 5'-monophosphate of adenosine, guanosine, cytidine and uridine together with the corresponding di and triphosphates, all occur in the three state in the cell and may be extracted with dilute acid.

Mono--, di-and triphosphates of pyrimidine deoxyribonucleosides have been found in acid extracts of thymus and other tissues, and the di and triphosphates of all four deoxyribonucleosides may be formed from the corresponding monophosphates by biological phosphorylation.

Coenzyme nucleotides—Many important biological compounds have nucleotide structures. They include coenzyme such as the nicotinamide nucleotides, flavin-adenine dinucleotide and coenzyme A, which are complex derivatives of adenosine monophosphate (AMP). The uridine nucleotide coenzymes play a part in the interconversion of sugars, CTP (cytosine triphosphate) is important in the biosynthesis of proteins and the adenine.

In this way the nucleic acid is actually composed by the chains of nucleotide units. A nucleotide unit consist of a molecule of sugar, a base and phosphric acid.

Now we have the question how the nucleotides are tied together in the polymer ? We have much information from the different sources and evidences that, however, both, DNA and RNA are linear unbranched polymers of nucleotides linked together by phosphate groups. The different nitrogen bases are attached with sugar units. The figure 123 represents sample chains or polynucleotides of the DNA and RNA type.

Deoxyribonucleic acid (DNA)—DNA is a polymer, the monomere units of which are the deoxyribonucleoside monophosphates. Most of the DNA's have high molecular weight, varying from 16^6

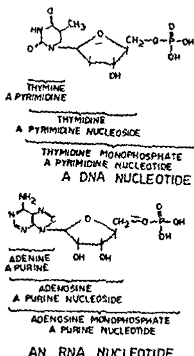


Fig. 120. Showing the structure of nucleoside and nucleotides.

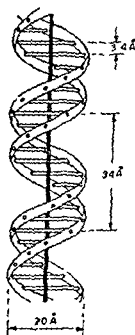


Fig. 121. Diagrammatic representation of the DNA molecule as proposed by Watson and Crick.

to 10^9 or more. DNA exists in cell nucleus in the form of deoxyribonucleoprotein and may be isolated by extraction from the cell followed by separation from the associated protein. The nucleoprotein complex may be extracted in molar sodium solution. The such obtain solution of DNA is purified severally.

It was Chargaff (1950, 1951) who first of all draw the attention to certain regularities in the composition of DNA. The sum of the purines is equal the sum of the pyrimidines ; the sum of the amino bases (adenine and cytosine) is equal to the sum of the keto (oxo) bases (guanine and thymidine) ; adenine and thymine are present in equimolar amounts, and guanine and cytosine are also found in equimolar amounts. The equivalence of A and T and G and C is of the utmost importance in relation to the formation of the DNA helix.

DNA's fall into two main classes, the 'A-T type' in which adenine and thymine are in excess and 'G-C type' in which guanine and cytocine predominate. In most DNA's only

the bases A, G, C and T are found but in some types of DNA 5-methylcytosine may replace cytosine to a limited extent. It is particularly abundent in the DNA of wheat germ. 5-Hydroxymethyl cytosine replaces cytosine in certain strain of bacteriophage.

Dotty (1961) has emphasized that the DNA molecule has only two basic physical properties, its molecular weight and its composition in term of the relative proportions of A-T and G-T pairs. The G-C pair is stronger then the other in the sense that it contributes more to the stability of the DNA helix. It also confers a

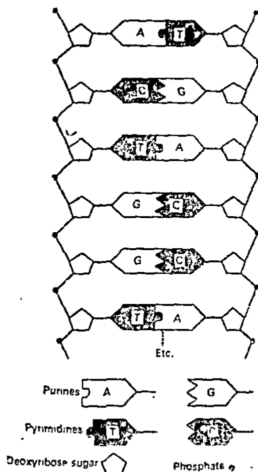


Fig. 122 The arrangement of the parts of DNA according to the Watson-Crick Theory. (A, adenine; G, guanine; T, thynine; C, cytosine.

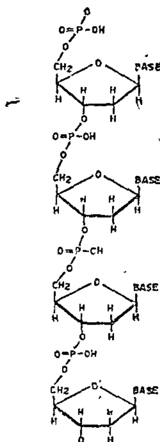


Fig. 123. A part of polynucleotide chain in DNA.

higher density on the DNA than does the A-T pair, as determined by gradient centrifugation.

The Structure of DNA—According to Levene, the DNA molecule has a long unbranched chain structure. It can be shown as in *the figure 126 as a polynucleotide chain* with 3′,5′ phosphodiester linkages.

The molecular Configuration of DNA—X-rays diffraction has been extensively employed in the study of tae molecular architecture of DNA, initially by Astbury (1947) and later by Franklin and Gosling (1953) and on a very extensive scale by Wilkins and his colleagues (1953, 1963, and 1964).

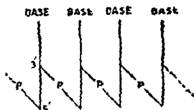


Fig. 124. A part of polynucleotide chain in DNA.

Such investigations have shown *that DNA gives two X-rays* patterns. When the water content is about 40 percent the *pattern is crystalline indicating the presence of three-dimensioual order.* At higher water contents a paracrystalline pattern is obtained indicating *that the molecules are parallel to each other but packed side by side* in a less regular manner. The same pattern have been obtained *with DNA's from several different sources. The crystallographic* repeat distance in the fibre direction is 28A° in the *crystalline* form and 34A° in the paracrystalline form *while the maximum possible* repeat distance in the fully extensive chain is only 7A° from phosphate *to phosphate group. This possibilities, however, indicates* the presence of several chemical repeats of the phosphate sugar chain *in one structural repeat.*

To account for these and other observations, Watson and *Crick (1953)put forward the view that the DNA molecule is a double* right handed helix consisting of two polynucleotide chains winding round the same axis and held together by *their bases. They* were, however, able to show, by making scale models, that the bases could *fit in if they were arranged in pairs of one purine and one pyrimidine,* and when the formation of the hydrogen bonds between the bases *was considered in detail it became evident that the only pairs* which would fit were adenine with thymine and guanine with cytosine and *they are bound with the hydrogen bond.*

As already been mentioned the Chargaff (1950) view about the

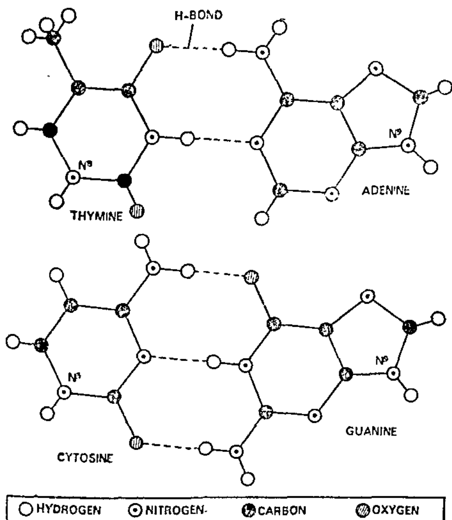


Fig. 125. Showing the hydrogen bonding between bases.

equivalence of adenine and thymine and of guanine and cytosine in DNA's from many sources. This, of course, is a most important piece of supporting evidence for the existence of the double-helical structure. The way in which the bases might be arranged in which a structure as shown in the figure 125 which shows the linkage by hydrogen bond of adenine in one chain and thymine in the other, or vice versa, and of guanine in the first chain to cytosine in the other, or vice versa. Accordingly, the order in which the bases occur in one chain automatically determines the order in the other chain, which is its complement. Apart from this essential condition, there are no restrictions on the sequence of pairs of bases along the

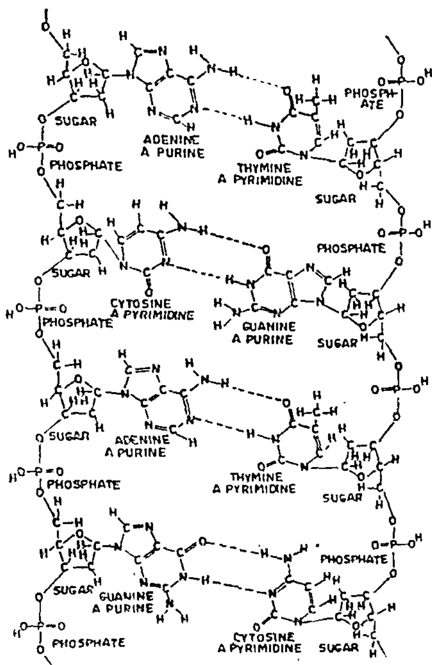


Fig. 126. A molecule of DNA. (According to Crick and Watson).

chains. The pairs of bases are flat and may be stacked one above the other like a pile of plates.

The systametic diagram of the Watson and Crick model is shown

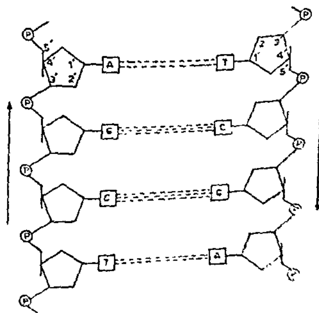


Fig. 127. Diagramatic representation of part of a hypothetical polynucleotide chain in DNA.

in figure 121 in which the two ribons represent the phosphate sugar chains and the pairs of bases holding them together are shown by the horizontal rods which form, as it were, the threads of a spiral stair case. Further, the finer details of the structure put forward by Watson and Crick has been corrected by Wilkins and his colleagues (955 and 1957), although the basic concept remains unchanged.

Watson and Crick pointed out, that in order to have maximum symmetry, the two strands must run in opposite direction. Further the double stranded structure is stabilized by the nitrogen bases which point toward each other and are capable of forming hydrogen bonds. The two strands of the double helix, formed between the base of purine and pyrimidine, because there are not enough place for two purine or for two pyrimidine. As it is quite clear from the figure No. 94 that only purine and pyrimidine pairs capable of forming hydrogen bonds are adenine-thymine (two hydrogen [illegible] and guanine and cytosine (three hydrogen bonds). Thus the [illegible] and C=G equally suggest a double helix as evident by [illegible] observations. Further the double helix, provides no [illegible] the sequence in which the base pairs follow each other [illegible] indicates clearly that DNA molecules are quite clear [illegible] architecture, only differing in specific sequence of [illegible]

Much evidences have been collected in recent years to make the Watson and Crick hypothesis one of the most firmly entrenched generalization of biological theory. All evidences received from (i) extensive X-rays deffraction studies, (ii) direct visualization with the electron microscope, (iii) its melting point, (iv) enzymatic digestion studies and by the Kornberg work of synthesis DNA in the test tube point out the Watson and Crick hypothetic double helix model of DNA.

Single-Stranded DNA and Circular DNA—One of the most interesting and unusual forms of DNA is that was isolated by Sinsheimer in 1959 (1959, 1959 and 1962) from a small virus ϕX 174 which attacks *E. coli.* The DNA molecule contains 5,500 nucleotide in a single strand. The conclusion that this DNA is single stranded comes from several lines of evidences :—

(1) It does not show the phenomenon of helix coil translation on heating. (2) It is attacked by a phosphodiestease from *E. coli* which acts superficially on single-stranded DNA. (3) It reacts with formaladehyde . double stranded DNA does not do so because the amino groups of its bases are protected by the strongly hydrogen bonded structure of the double helix. (4) Its molar prepartions of bases do not show the equivalence of A and T and G and C required for double helix formation.

Subsequent evidences, however, suggested that when ϕX 174 DNA is prepared by very gentle extraction, it is not merely single-*stranded but that the strand is closed on itself to form a ring.* For example since it is not readily attacked by phosphodiesterases from *E. coli* or spleen it can have no free 3'-hydroxyl termini nor is its susceptibility to these enzymes increased by pretreatment with phosphomonoesterase which would remove terminal phosphate groups. Moreover ultracentrifugation reveals two components, a fast moving component (S_1) which is infective and a slow moving component (S_2) which is not infective. Further, the careful treatment of S_1 with pancreatic deoxyribonuclease converts it to S_2 with loss of infectivity but with no significant lowering of molecular weight. More drastic treatment, however, leads to the formation of further degradiation products. The results suggest that the S_1 component is a covalently linked ring structure which yields an open-chain *degradiation product* S_2. Further other viral DNA's can assume a circular configuration include the double-stranded replicative form of bacteriophage ϕX 174, the DNA of bacterophage lamoda and

the DNA of polyoma virus which exists in a ring form straight chain form. Both forms are double-stranded and ..... are infective. The ring structure is evident in electron micrograph. The DNA of T_2 bacteriophage can readily be made to assume a circular form. A peculiar form of single stranded DNA is found in association with yeast lactic dehydrogenase (YLDH DNA). It is composed of 33 nucleotide units per enzyme particles.

Properties of DNA

(1) Modern methods of preparing DNA yield fibrous solids, resembling asbestos. Their sodium salts form viscous solution on which the high asymetry of the molecule confers certain characteristic properties. For example, such solution readily form gels and exhibit the phenomenon of negative streaming birefrigence or double refraction of flow. Further when the fluid is at rest no light passes; when it is stirred, the light is able to pass through.

(2) The molecular weight of DNA is usually determined by light scattering measurements or by measurements of sedimentation rate or intrinsic viscosity, and value of about 10^6 are frequently quoted. The value, however, may be too low, for the DNA threads are fragile and are easily susceptible to hydrodynamic shearing forces. Even the stirring or pipetting of a solution many result in breakage of the chains. It is however, believed that the DNA molecule as it exists in the natural state in the cell may be much larger than was hitherto suspected. Further confirmation of this view comes from the studies on the DNA T_2 bacterophage.

(3) One of the most important properties is its behaviour on denaturation by heating. At temperature about 5C° above T_m, the two strands of DNA helix come apart through Brownian movement. If the solution is cooled repidly the two single strands remain separated, but if it is cooled slowly specific recombination of the two strands may occur and the double helix is restored.

Nucleotide Sequence—However, the determination the sequence of nucleotides along the DNA chains has become all the more urgent in the knowledge that the order in which the bases occurs spells out the genetic message or code. Although problem of determining nucleotide sequence in DNA resembles that of determining amino acid sequence in protein, but is immensely more difficult. The reason for this lies partly in the difficulty of obtaining a single molecular species of DNA from most natural sources and partly in the fact that the DNA molecule is a very large polymer composed of

only 4 different monomer types. Moreover, the methods available are not as refined as those used for work on protein. The sequences of nucleotide have been used in details in the chapter synthesis of nucleic acid.

Biological significance of DNA—It is awell known fact that the genetic or hereditary material of the cell must have two separate functions. Firstly it must be capable of self duplication and secondly of initiating actions that ultimately find expression in a given cell structure or function. With the help of biochemical genetics, it is new clear that the expression of gene action is the formation of a protein. In this way DNA must therefore be capable for both of the functions, *i e*. duplicating itself and for providing necessary informations for protein synthesis. It is obvious that the structure of the DNA provides a convenient device whereby a particular molecule with a particular sequence of base pairs could determine the lying down of a "complementary strand" resulting in the formation of two identical molecules. **Levinthal** and **Crane** suggested a precise geometrical model whereby the duplication of DNA molecule could occur in a continuous without requiring the prior separations of strands. They further suggested that the duplication begins at one end. It provides the energy to open up the strand for the rotation of the two lengthening daughter strands as well as shortening them. It should be kept in mind here that enough energy was available to over come viscous drag opposing these rotations.

The role of DNA is to store and to transmit information, to be used in protein synthesis. It would appear that the specific sequence of the bases along this linear structure, provides the code necesary for the determination of protein structure.

Ribonucleic acid—RNA is the polymer, the monomer units of which are ribonucleoside monophosphotases. The living cell, whether from mammalian, bacterial or other sources contains three main kinds of RNA :—

(a) Ribosomal RNA or (r-RNA).

(b) Soluble, 'transfer', or acceptor RNA (s-RNA).

(c) Messenger RNA (m-RNA).

(a) **Ribosomal RNA**—The bulk of this type of RNA is about 80 percent and is contained in the minute cytoplasmic particles known as ribosomes. Ribosomal RNA (r-RNA) is of high molecular weight (0 5—2·0 $\times$ 10^{5}) and is metabolically stable, Ribosomal

RNA constitutes the bulk of the cellular RNA. In bacteria, ribosomes contain about 60 percent protein and 40 per cent RNA. Ribosomes are the exclusive site of protein synthesis, but until recently we believed their only role was to orient the m-RNA and t-RNN. Each ribosome is quite large with a molecular weight near 3 million. They are constructed of two subunits, one having the molecular weight of 1·8 million and the smaller about half of that weight (0 9 million). Ribosomes and their submits are often described in terms of Svedberg (S), which are a measure of the speed at which a particle sediments in the ultracentrifuge. The two subunits mentioned above are 30S and 50S ; in combination they have a sedimentation constant of 70S. The large 70S ribosome falls apart when Mg^{++} concentration reduced. Both subunits contain both protein and RNA. Their further dissociation results in 16S and 23S r-RNA units which are probably the fundamental ribosomal structural units. It should be noted that some what larger units of 18S and 28S are characteristic of fungi and higher organisms.

It has been postulated (though conflicting report exist in the scientific literature) that ribosomal RNA may perform an m-RNA like function in the production of its own structural proteins, then fold up into completed ribosomes. It is known that the RNA form a completed ribosome cannot serve as a messenger, however. It nascent r-RNA can serve such a role it must be altered (*e. g.* by the addition of methyl groups to certain bases) as the ribosome is constructed. The presence of RNA in ribosome suggests some sort of base pairing. However, the precise function of ribosome r-RNA is unknown. There is some recent evidence that one of the subunits of r-RNA serves to release the m-RNA from DNA.

(2) Transfer RNA—A group of small RNA molecules exist that serves as acceptors of aminoacids. At least one t-RNA has a slightly different structure. However, the t-RNAs studied have great similarity in size, each containing seventy-five to eighty nucleotide with a total molecular weight of approximately 25,000. They occurs as single chains, but a great deal of hydrogen bonding is present, perhaps due to the chain fold back on itself in a cloverleaf shape. These region of base pairing however, resemble a DNA double-helix in that they assume a coiled configuration. Another characteristic point of t-RNA is the chain endings, which are identical for all t-RNA types. One chain end, with a 3′-hydroxyl group, always terminates with the three bases CCA. The other end of the chain

(with a 5'-phosphate) terminates with G (guanytic acid).

The t-RNA is the unique in that it contains bases other than the four commonly found in RNA (A, U, G and C). One such base is pseudouridylic acid, in which the ribose is attached to the 5-carbon of uracial rather than to the 3-nitrogen. A numbers of other unusnal bases have additional methyl groups attached. Frequently, these methyl groups interfere with base pairing when they replace hydrogens involved in hydrogen bonding. These regions of t-RNA containing methylated bases could not exist as double-stranded regions and may, infact, exist specically to prevent such base pairing. The unusual bases are probably formed after the usual base sequeece are laid down. How enzymes determine which base is to be methylated is far from clear.

Holley (1964) performed the difficult analytical job of determining the nucleotide squence of the particular t-RNA in yeast that specifically binds the aminoacid alanine. This t-RNA has seventy seven nucleotide and is characterized by the presence of several (nine) unusual bases. Until Holley's investigation, it was felt that the secondary configuration of t-RNA would be obvious if the sequence was known. However, several alternative steps are possible to allow a fair amount of hydrogen bonding.

Even though t-RNA is the smallest of the nucleic acids, but plays a key role in protein synthesis. However, need for three or four specific rrgions or sites in the molecule can be recognised. One is the aminoacid attachment site, which occurs on one end of the molecule and is the same for all t-RHAs and consists of the base adenine (A). A site must however, exist by which the aminoacids is attached is the correct t RNA though the action of a specific enzyme synthetase). Suca a site is called the *recognition site* and must match the enzyme in some way : otherwise t-RNA would be changed with the wrong aminoacid. The third site is the anticodon triplet of bases which is complementary with the piplet of m-RNA Probably these bases would occur in the impair (single-stranded) region of the loop of t-RNA. It is thus fact, that the recognition site and the anticodon are identical.

Messenger RNA (m-RAN)—Studies by Hershey and latter by Volkin and Astrachan) with T_2 bacteriphage-infected (bacterial virus) *E. coli* in (1956) led to the first existance for the unique kind of the RNA, later to be called messenger RNA or m-RNA. They however, noted that this infection, the RNA formed a as similar in base ratio

(with U substituted for T) to that of phage DNA—but unlike the DNA of the host bacteria or the bulk RNA,

The recent studies have shown that the formation of m-RNA is not unique to infected cells but constitutes 2 to 4 percent of the total RNA of normal cells. m-RNA is markedly unstable in bacterial system but far more stable in higher organisms. This characteristic of repid turnover plays on important role in genetic regulation. If m-RNA serves as an intermediate in information transfer from DNA, its rate of formation and breakdown can impose a subtile mode of control on the expression of particular genes. With certain experiments done on the configuration of the m-RNA suggests that m-RNA is identical in base sequence (except for U for T) to one DNA strand and is complementary to its particular strand. Each m-RNA molecule has a nucleotide composition and sequence that is complementary to that of the DNA template on which it was formed. The nucleotide sequence of an m-RNA molecule endows it with the information necessary for ordering aminoacids into their proper sequence.

The structure of RNA—The most important preliminary consideration is the nature of the inter nucleotide link. Alkaline fission of RNA results in neutralization of alkali ; and thus it is clear therefore that some or all of the phosphoric acid groups are involved in the nucleotide linkage. Since the intect nucleic acid may be deaminated by nitrous acid, the amino groups do not take part in the linkages ; neither do the hydroxyl groups of guanine or uracil, for electrometric titration reveals that they are unsubstituted. The early work of Gulland and Jackson suggested the involvement of

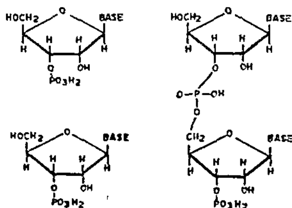


Fig. 128. Base structure and formation of nucleoside in RNA.

C-5′ and this has been confirmed by Cohn and Volkin (1953), wh treated RNA with diesterase from snake venom and obtained mixture of 5′-phosphates of all four nucleosides. On the othe hand, digestion with alkali gives a mixture of nucleoside 2′-anc 3′-phosphates. It would appear thus that the main internucleotide linkage are phosphoester group connecting C-5′ in one nucleotide with C-2′ or C′-3 in the next nucleotide.

It is however, theoretically possible that such a structure could carry side chains attached either at C-2 or at triply esterified phosphate groups, but the general consensus of openion is against the occurence to any appreciable extent of branched chain

Further as pointed out by Todd and his Collaborators (1952 and 1953), that alkaline hydrolysis yield two isomeric forms of each nucleotide, originally termed as a and b nucleotides but sub-

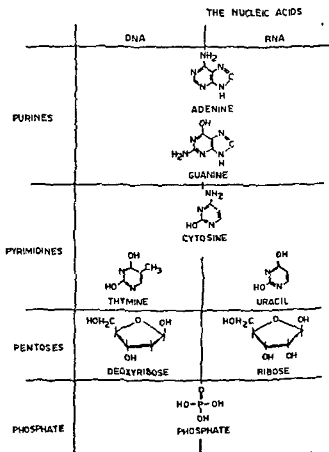


Fig. 129. Structure of DNA and RNA.

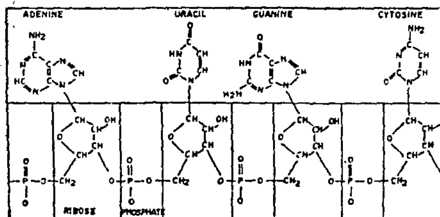


Fig. 130. Structure of ribonucleic acid.

sequently proved to be the nucleoside 2′-and 3′-phosphate respectively. These isomers are readily converted into a mixture of both under acid conditions but are stable without interconversion in alkaline solution. This interconversion, however, involves the formation of a cyclic intermediate, the nucleoside 2′, 3′-phosphate, which yield on hydrolysis a mixture of the 2′- and 3′-phosphates.

Molecular weight of RNA—The molecular weight of RNA's can be determined by physical measurements of sedimentation, diffusion constent and intrinsic viscosity or light scattering. The value obtained by different authors show considerable variations.

The most consistent results are obtained with s-RNA which appears on gradient centrifugation in the 4S region and show values for the molecular weight between, 23,000 and 28,000. Similar values are given by chemical determination of molecular weight based on end group analysis.

Ribosomal RNA falls into two main groups. In *E. coli* the 50S and 30S ribosomes yield RNA's of moleculur weights $1{\cdot}12\times10^6$ (23S RNA) and $0{\cdot}56\times10^6$ (16S RNA) respectively. It has been suggested that these two RNA's are built up from multiple of a single subunit and that the 125 RNA is a dimer of 16S RNA, but this conclusion is rendered unlikely by the observation that the two RNA's differ in base sequences and can hybridize with different sites on the bacterial genome. Molecular weights of at least 2×10^6 are found for the RNA's of certain viruses, such as tobacco mosaic virus. Further the figure in the literature available for the molecular weight of m-RNA show wide variations. In general the value appears to be $0{\cdot}5\times10^5$ or greater.

Secondary structure of RNA—While DNA has a firmly established helical structure, the nature of the secondary and tertiary structure of RNA is less well defined but has already been partially established. In solution of low ionic strength RNA molecules behave the typical highly swollen polyelectrolyte chains, but an increase in the ionic strength causes the chains to contract upon themselves so as to display relatively low intrinsic viscosities and high sedimentation rates. This however suggests the existance of base pairing in certain regions of the RNA chain such as is known to occur in some of the biosynthetic polyribonucleotides.

Further under the influence of the enzyme polynucleotide phosphorylase, biosynthetic polynucleotide may be obtained with some interesting features. If the substrate employed is adenosine diphosphate (ADP), the polymer formed is a ribopolynucleotide containing adenine as its only base. It is usually referred to as poly A. Poly U may be formed similarly from UDP as substrate, and if the substrate is an equimolar mixture of ADP and UDP, the product is poly AU.

When equimolar amount of poly A and poly U are mixed in dilute aqueons solution they form a complex known as poly (A) : (U) in which the adenine moieties of one strand are linked by hydrogen bonds to the uracils of the complementary strand. The X-rays diffraction puttern of this complex indicates a double helical structure, as in DNA, with ten base pairs per turn of the helix, the pitch of which is 34A°. The formation of helical complex is accompanied by a 34 per cent depression in the absorbance at 260 m μ below the value for the sum, of the two constituents (the hypochromic effect). The helical complex behaves in many ways like DNA. It shows the phenomenon of 'molecular melting' or helix coil transition.

All these features are consistent with a relatively rigid secondary structure involving two complementary limbs linked by hydrogen bonding between A and U and between G and C. The helical conformation of the molecule is confirmedly X-rays analysis.

The chemical synthesis of Ribopolynucleotide—Khorana and his colleagues (1963) have devised chemical methods for the specific synthesis of the (C-3')—(C-5') internucleotide linkage and have applied them to the synthesis of ribopolynucleotides both by stepwise procedure and by polymerization procedure. For example they have succeeded in synthesizing dinucleotides of the type p AUp and Cp Up, the tetranucleotide Up Ap Up U, and

homologous adenosine polynucleotides.

Nucleases and Related enzymes—The enzymes which hydrolyse polynucleotide chain can all be regarded as phosphodiesterases in the sense that they break the phosphodiester internucleotide linkages but they can readily be subdivided into two main groups :—

(1) The *endonucleases* which attack linkages in the interior of the nucleic acid chain and break it into fragments which may vary in size from mononucleotides up to acid-precipitable polynucleotides. They are sometimes referred to as nucleodepolymerases or nucleophosphodiesterases.

(2) The *exonucleases* which attack polynucleotide by the consecutive splitting of mononucleotides from one end of the chain. These enzymes are frequently referred to simply as *phosphodiesterases.*

Endonucleases—The endonucleases are divided into two main groups, the *ribonucleases* which attack RNA and *deoxyribonucleases* which attack DNA. Various forms of ribonuclease (RNase) have been isolated from different sources but the best is that derived from pancreas. Pancreatic ribonuclease enzyme is usually prepared from fresh beef pancreas by extracting the glands with ice cold 0·25 N-sulphuric acid and precipitating with ammonium sulphate.

Further there are two main type of deoxyribonuclease (DNase) which have been fairly well characterised ; both are endonucleases The first type is the pancreatic deoxyribonuclease (DNA I) is a

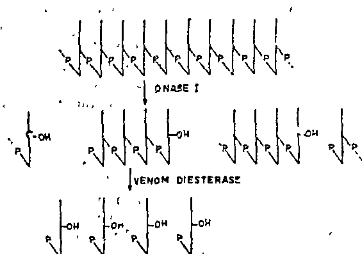


Fig. 131. Showing the digestion of DNA by Dnase I,

Secondary structure of RNA—While DNA has a firmly established helical structure, the nature of the secondary and tertiary structure of RNA is less well defined but has already been partially established. In solution of low ionic strength RNA molecules behave the typical highly swollen polyelectrolyte chains but an increase in the ionic strength causes the chains to contract upon themselves so as to display relatively low intrinsic viscosities and high sedimentation rates. This however suggests the existance of base pairing in certain regions of the RNA chain such as is known to occur in some of the biosynthetic polyribonucleotides.

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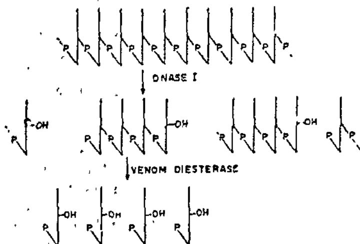


Fig. 131. Showing the digestion of DNA by Dnase I,

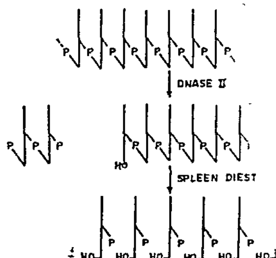


Fig. 132. The digestion of DNA by DNas

5'-phosphomonester former. The second type (DN
found in spleen and thymus is a 3'-phosphomonoest

Not only this, but DNA-specific exonucleases have been noted. These enzymes are of general int mode of action has been worked out in some detail. of three types.

(a) E. coli exonuclease I.

(b) E. coli exonuclease II.

(c) E. coli exonuclease III.

Phosphomonoesterase—These enzymes remove as ino throphosphate, the terminal monoestrified phosphate gro mononucleotides or oligonucleotides. The 5'-nucleotidase have been prepared from seminal plasma and snake venom the phosphate group from nucleoside 5'-phosphate. The a phosphatase from *E. coli* hydrolyses a wide range of comp containing monoesterified phosphate.

SUMMARY

About 100 years ago a Swiss biochemist, Friedrich Miescher, noted the presence of non protein stuff in the cell, to which he gave the name nuclein. Later it became known as nuclei acid. At the beginning of the twentieth century nucleic acid was found to include nitrogen-containing compounds called bases.

The nucleic acids play the major role in the living system. Each nucleic acid is made up of many nucleotides. The each nucleotide has three components: (1) a pentose sugar, (2) phosphate, (3) a base. Deoxyribonucleic acid (DNA) contains the pentose sugar deoxyribose and ribonucleic acid (RNA) contain ribose sugar. The four bases in DNA are adenine, guanine, cytosine, and thymine. They are same in the RNA, with the exception that uracil replace thymine.

DNA is double-strand helix. The strands are formed by the deoxyribose units. They are held together in the helix by the hydrogen bonding between base pair. Each half turn of the helix contains five pairs. During cell division, the helix unwind, the bonds are broken and each of the two strands forms a new complementary strand of DNA. For protein synthesis, each strand of DNA directs the formation of a strand of RNA with complementary base. This RNA, called m-RNA or messenger RNA. The rest two RNAs are the transfer RNA or t-RNA and ribosomal RNA or r-RNA. They all help in protein synthesis.

Several enzymes such as endonucleases and exonucleases, however, act to hydrolyse, the nucleic acid into their respective components. They are, however, very useful to the study of configuration and chemical structure of nucleic acids. Phosphomonoesterase enzymes always act to remove the inorganic orthrophosphate, the terminal monoesterified phosphate group from mononucleotides or oligonucleotides.

NUCLEIC ACID SYNTHESIS

The study of the biosynthesis of the nucleic acids involves the due consideration of several important aspects such as biosynthesis of purine and pyrimidine ring systems, the origin of sugar components and the biosynthesis of the polynucleotides themselves.

The Biosynthesis of Purines—Liver is the primary source of purines in mammals. The origin of atoms comprising the purine skeleton is illustrated in the figure 133. It can be observed from the figure that the purine skeleton is synthesized from small fragments N-1 is derived from amino group of aspartate, N-3 and N-9 from the amide nitrogen of glutanine, C-2 and C-8 from "fermate" (carried by the coezyme of the tetrahydrofolate type), C-6 from CO_2

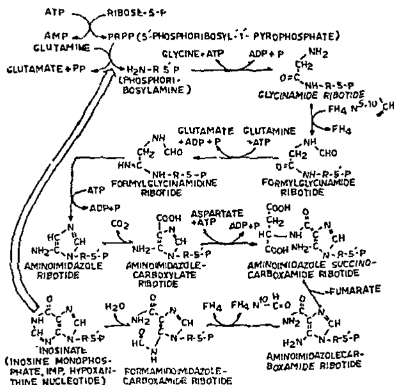


Fig. 133 Synthesis of purines.

and where as the sequence C-4 ; C-5 ; N-7 is derived from glycine. The synthesis of the purine, however, begins with the formation of phosphorisyl pyriphosphate. This is converted into amino sugar, by reaction with glutamine. The amino sugar then condenses with glycine to form glycinemide ribotide, which is then is formylated by anhydroformyl-tetrahydrofolate. After this, the formylglycinamide ribotide is aminated to formylglycinamide ribotide by reaction with glutamine. Closure of the ring and reaction with molecule of CO_2 produce aminoimidazolecarboxylate ribolide. The compound is converted to an amide by condensation with asparate and cleavage to fumerate from the product aminomidazolecarboxymide ribotide which is formed by the condensation is onward formylated to a formamide derivative, the formyl carrier in this case being formyl-tetrahydrofolate. Cyclization of the remaining ring forms inosinate, the parent compound of all the purine.

However, the isosinate can be converted into adenylate and guanylate components of the nucleic acids as given in figure 134.

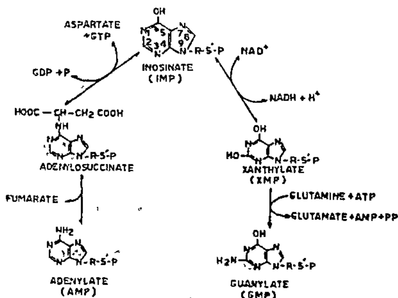


Fig. 134. Inter conversion of purine into different purine nucleosides.

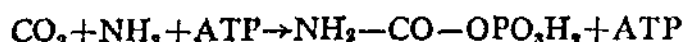
The adenylate can arise by amination of the keto-enol group position 6 of inosinate. The reaction however, involves condensation with aspartate and subsequent splitting out of fumarte. Guanylate is formed by oxidation of inosinate to xanthylate, followed by amination utilizing glutamine.

Our knowledge of the mechanism where by these components are assembled to form the purine ring is largely due to the work of Buchanan and his co-workers (1957) and the Greenberg and his collaborators (1957).

The biosynthesis of the pyrimidines—The biosynthetic pathway of the pyrimidine has been established mainly on work with micro-organism using the mutant technique, but the same system appears to operate in mammalian tissues.

Degradiation experiments with labelled pyrimidines have shown that in the synthesis of the pyrimidine ring, N-1 is derived from ammonia (NH_3), carbon-2 from CO_2 both by the way of carbamyl-phosphate. The chain composed of nitrogen 3 and carbons 4, 5 and 6 are formed from aspartate. "Formal" is the source of methyl group of thymine in DNA. Incorporation experiments with labelled possible precursors have also revealed that ureidosuccinic acid and orotic acid(uracil-6-carboxylic acid) lie on the biosynthetic pathway.

The first step involves the interaction of CO_2 and ammonia under the influence of ATP form carbamoylphosphate (CP) in the presence of carbamate kinase.

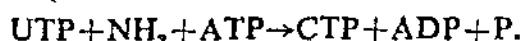


$$CO_2+NH_3+ATP \rightarrow NH_2-CO-OPO_3H_2+ATP$$

The carbamoylphosphate then reacts with aspartic acid under the influence of aspartate carbomyl transferase to form ureidosuccinate (US). Further under the influence of enzyme dihydroasotase which has been isolated by Lieberman and Kornberg (1954) ring closure of US is effected to yield dihydro-orotic acid (DHO) which in turn is however oxidised by dihydro-orotic acid dehydrogenase to yield orotic acid (OA).

Further under the influence of orotate phosphoribosyltransferase and Mg^{++} ions, OA form orotidine-5′-phosphate. (5′-OMP), which is then decarboxylated by orotidine-5′-phosphate decarboxylase to yield uridine-5′ phosphate (5′-UMP). An alternative pathway of pyrimidine biosynthesis have also been suggested through β alanine

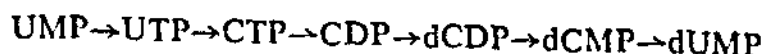
The biosynthesis of cytosine derivative—Conversion of uracil to cytocine takes place at triphosphate level under the influence of the enzyme CTP synthetase.



$$UTP+NH_3+ATP \rightarrow CTP+ADP+P.$$

The biosynthesis of deoxyribonucleotides—The conversion of rise to deoxyribose take place at nucleotide level with breakage of the glycosidic linkage, since such compounds as uniformly labelled C^{14}—cytidine are incorporated into the d CMP residues of DNA without change in the relative specific activities of sugar and base. This conversion appears to take place at nucleotide diphosphate level. Extracts of *E. coli* contain two enzyme fractions; one of which catalyses the formation of CDP (cystine diphosphate) from CMP (cystine monophosphate), while the second brings about the reduction of CDP to d CDP. For this reaction there is a requirement for ATP, Mg^{++} ions, NADPH and thiorodioxin, a low molecular weight acid protein consisting of a single polypeptide chain with one S-S bond (cystine).

It is most probable that d AMP and d GMP are also formed by reduction of the diphosphate level. On the other hand of UMP is produced by deamination of CMP under the influence of deoxycytidylate deaminase. The complete route for the conversion of UMP to d UMP would therefore be :—



$$UMP \rightarrow UTP \rightarrow CTP \rightarrow CDP \rightarrow dCDP \rightarrow dCMP \rightarrow dUMP$$

The biosynthesis of thymine derivative—The important step in the formation of thymine nucleotides is the methylation of deoxyuridine monophosphate (dUMP) to produce thymidine monophosphate (dTMP) (dUMP→dTMP) under the influence of an enzyme system referred as thymidylate synthetase. The process is elaborate and however occurs in several steps. Formate and β-carbon atom of serine have been shown by several authors to be the precursors

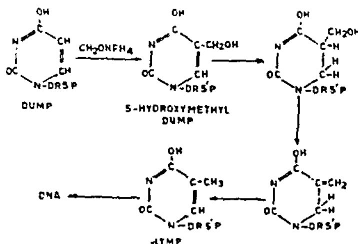
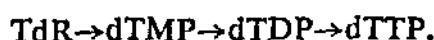


Fig. 136. Biosynthesis of thymine derivative and DNA.

of the 5-methyl group. Reichard (1960) and Friedkin and Roberts (1955) observed that C^{14}—deoxyuridine is incorporated into the thymine ring system. It appears that a deoxyribonucleotide (or nucleotide) is the one carbon accepter molecule of thymine biosynthesis.

The formation of nucleoside triphosphate—In the biosynthesis of RNA and DNA the substrates for appropriate polymerases are the ribonucleotide 5'-triphosphates and the deoxyribonucleoside 5'-triphosphate which are produced from the corresponding nucleotide monophosphate by the appropriate kinases which convert thymidine (TdR) to its triphosphate (dTTP). Thymidine is readily incorporated into cells which are synthesizing DNA and the incorporation of labelled thymidine have been very extensively studied on the biosynthesis of DNA. The incorporation of TdR into DNA

involves several stages in which it is converted by a series of kinases to dTTP by stepwise phosphorylation, as


TdR→dTMP→dTDP→dTTP.

The process of nucleotide biosynthesis is carried out under the control of elaborate system The formation of purine and pyrimidine ribonucleotides is controlled by well-known feed back mechanism.

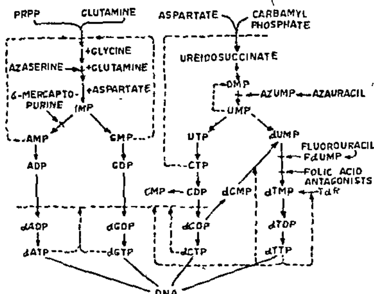


Fig 137. Pathway involved in the biosynthesis of deoxyribonucleoside triphosphate.

Further the biosynthesis of DNA can be considered as taking place in four main stages :

(1) The biosynthesis of purine and pyrimidine ribonucleosides monophosphates.

(2) The conversion of these ribonucleotides to the corresponding deoxyribonucleotides.

(3) The phosphorylation of the deoxyribonucleoside monophosphates to the triphosphate stage.

(4) The polymerization of the deoxyribonucleoside triphosphates to yield polydeoxyribonucleotide in the presence of an appropriate DNA primer. The process occurs as follows :—

Polycursors
↓
Ribonucleotides—e. g. AMP.
↓
Deoxyribonucleotides—e. g. dAMP, dTMP.
↓ Kinase
Deoxyribonucleoside triphosphate —e. g. dATP, dTTP
↓ Polymerase
DNA

Fig. 138. Stages of the biosynthesis of DNA.

Synthesis of DNA (Replication)—The currently accepted view of DNA synthesis is illustrated in the figure. Under the influence of DNA polymerase, in the presence of Mg^{++}, the double strands of DNA are believed to separate, a small portion at a time clearing the hydrogen bonds between complementary bases. Deoxynucleoside triphosphate, all four of which are required for synthesis to

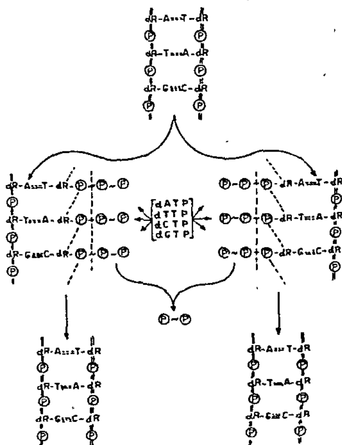


Fig 139. Replication of DNA.

proceed, are attracted from the solution in the nuclear sap to form hydrogen bonds with their complimentary bases on the separated strands. Each new nucleotide looses a pyrophosphate group while forming an ester linkage from its remaining phosphate to the 3′-hydroxyl group of the deoxyribose on an adjacent new nucleotide. The two daughter double helices are formed, each consisting of an old strand of the present DNA, coupled to a complementary new strand, the final composition and nucleotide sequence of each strand being identical with the corresponding strand which existed in the present molecule The process has been named as replication". Its suitability for the duplication of genetic information is evident. Although the present DNA, which must be present for synthesis to occur, has been called a "primer", its function differs fundamentally from that of the priming polysaccharide in glycogen synthesis. It would be more appropriate to use the term "template". This mechanism of replication is called semi-conservative.

DNA replication is however inhibited by acridine drugs, *e. g.* proflavine, probably by interaction of the inhibitor between successive base pairs, thus distributing the structure of the double helix. Antibiotics of the mitomycin yield similar result.

One theoretically possible mode of replication would involve the fragmentation of the DNA mulecule in such a way that the new daughter molecules contained sections of old DNA and a section of new DNA along each single strand. Each strand of DNA would, therefore, be a mixture of old and new. There is at present no evidence for this mechanism.

The mechanism of DNA biosynthesis—While it may be accepted that DNA strands are conserved during replication, the details of the mechanism where by the complementary strand is formed depend upon the study of enzymatic processes by which DNA chain are formed. Our knowledge of the *in vitro* enzymatic synthesis of DNA rests mainly on the work of Kornberg (1961) in bacterial system and of Bollum (1963) in a mammalian system. The original observation (Lehman, *et al*, 1958) was that crude extracts of *E. coli*, when incubated with radioactively labelled deoxynucleoside triphpsphates, gave rise to a trace conversion of the soluble triphosphate into acid insoluble products. The result was interpreted that a small amount of DNA had been synthesized, but that most of the product was broken down in these crude extracts by the nucleases that were undoubtedly present. Kornberg visualized the problem as one of the enzyme purification, he and his colleagues, however, separated

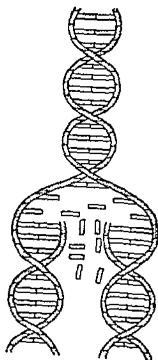


Fig. 140. Showing the gene duplication according to the Watson-Crick Theory.

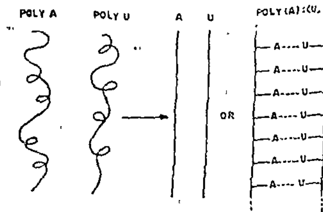


Fig. 141. Showing the association of single strands of poly A and U to form the poly (A) : (U) complex.

DNA polymerase from contaminated nucleases. The enzyme is now several thousand fold purified. Similar enzymes exist in other bacterial cell and in animal cell.

This enzymatic biosynthesis can only be brought about in the presence of all the four deoxyribonucleoside triphosphates (A, G, T, C). DNA must be present as a primer. The DNA can be from *animal, plant, bacterial or virusus sources. The primary DNA must* have a high molecular weight in order to be obtained to the extent of twenty times or more of the weight of the primer *added, until* one of the substrates is exhausted. The organic phosphate is however released as shown in the figure. If even one substrate is omitted, the extent of the reaction is diminished. Significant *incorporation* is still obtained, but this amounts to very little and is due to the *incorporation of a few nucleotides at the end of the primer chains,* This so called "limited reaction" is also governed by base pairing rules in the sense that the DNA priming molecules may have ends of slightly unequal length. It is thought *that in*

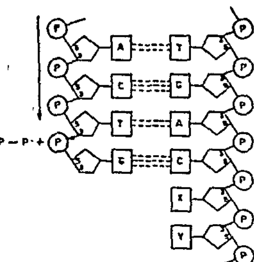


Fig. 142. Mechanism of enzymatic DNA replication.

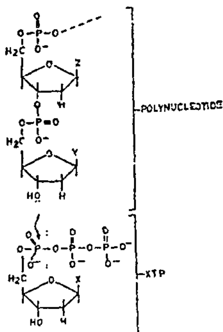
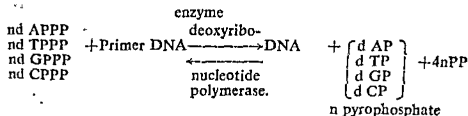


Fig. 143. Probable mechanism for expending a DNA chain.

such a situation the shorter chain might add a few nucleotides complementary to the nucleotides found in the longer sister chain, but there is no evidence for this. If we omit the primer DNA, there is no synthesis at all. Presumably the necessary template is missing.

In the process all the triphosphates are act primer DNA. In the presence of Mg^{++} ions and the enzyme **deoxyribonucleotide polymerase**, when all these substrate were mixed, the DNA production occurs in the following way.



Many enzymes act by breaking the free added nucleotide units into DNA strand. Various phosphodiesterases enzyme could break the chain at specific point. Thus it seems that DNA m olecule

grows by the addition of nucleotide to the hydroxyl group (–OH) at 3 carbon position deoxyribose. Further investigation by Josse and Kaiser (1961) demonstrated that polarity of the strands of DNA is of a particular importance.

Further the DNA produced *in vitro* by Kornberg's enzyme has a base composition that shows A=T and C=G, as did the primer DNA.

The enzymatic biosynthesis of the DNA may be summarised in the following steps :

(1) The formation of the deoxyribonucleoside monophosphate.

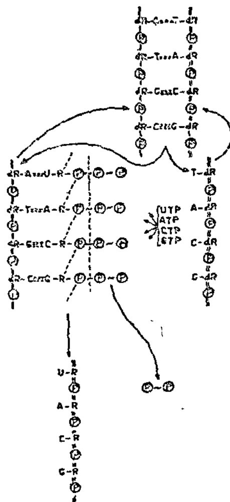


Fig. 144. Probable mechanism of transcription of DNA to RNA.

(2) Then their phosphorylation to the triphosphate stage by appropriate kinases.

(3) The polymerization of the triphosphates to DNA by the action of the polymerase (nucleotidyltransferase) in the presence of Mg^{++} and the appropriate primer.

The mechanism of the synthetic reaction involve a nucleophilic attack on the pyrophosphate-activated deoxyribonucleoside 5′-phosphate by the 3′-hydroxyl group at the growing end of a poly deoxyribonucleotide chain. The inorganic phosphate is liberated and the chain is lengthened by one unit. As might be expected the reaction is inhibited by pyrophosphate.

Synthesis of RNA (Transcription)—The synthesis of RNA is more complex than that of DNA. There exist several types of RNA, each varying in quantity (and, in certain case, composition and nucleotide sequence) with the physiological state of the cell,

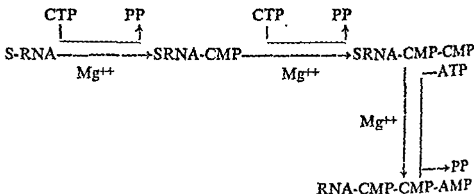
which is in turn is affected to many environmental influence. The general pattern of RNA synthesis is energing from recent investigations, making it possible to sketch a probable mechanism as shown the figure.

The recent evidences suggest that virtually all of the RNA of the animal cell is synthesized in the nucleus, although most of it eventually finds its way into the cytoplasm. The exception only occur in mitochondrial RNA, which appears to be synthesized within the mitochondria under the influence of small amount of DNA found in these organelles. Further, recent evidences suggest the view that all RNA is synthesized on a DNA template. Since this process amount to the transfer of the information contained in the DNA over to another species of nucleic acid, it is however appropriately termed "transcription".

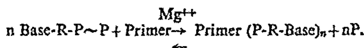
It is however just clear from the figure that the beginning of the process resembles DNA replication, *i. e.* some degree of separation of the double strands of DNA and cleavage of hydrogen bonds must occur. Since there are certain evidences from bacterial genetics that much (in not all) of DNA directed RNA synthesis is blocked by repressor substance if not in action, this separation of strand thus need the service of de-repressor in addition to the enzyme, RNA polymerase. In any case only one of the two strands of DNA is active in transcription in vivo, although it is not known how this is accomplished. In the presence of Mg^{++} and the polymerase, nucleoside triphosphates (all four of which must be present) are attracted to the solution, their bases forming hydrogen bonds with complementary bases on the transcribing strand of DNA. The process and the subsequent union of ribonucleotide to form RNA are analogous to DNA replication, the major difference being the type of pentose involved and the substitution, in the case of RNA, of uracil for thymine in base pairing in adenine.

Further, no processing appears to be required in the synthesis of that species of RNA which specifies the sequence of amino acids during protein synthesis, massenger RNA (m-RNA). Two types of ribosomal RNA (r-RNA) of different molecular weights and of many small molecules of the RNA species.which carries activated amino acids (soluable RNA, s-RNA ; transfer RNA,. t-RNA), are partially methylated in the nucleus (various base-specific RNA transmethylases), and also many undergo certain rearrangements or other modification of the usual bases (forming hypoxanthine

or dihydrouracil). Further any molecule of S-RNA deficient in the terminal sequence cytidylic-cytidytic-adenylic, required for transport the amino acids, can acquire these terminal nucleotides by the following sequence

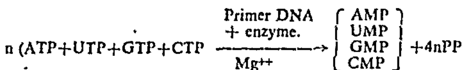


The bacterial enzyme, the polynucleotide phosphorylase, calalyzes the following reactions.



Further it is an established fact that in all the synthesis different kinds of RNA run under the direction of DNA. The demonstration by Ochoa and his collaborators about the synthesis of RNA is only now a questionable biological significance. In 1955 Grunberg, and manago Ochoa isolated an enzyme called ribonucleotide phosphorylase from several microorganisms such as *Azotobacter vinelandii* and *Escherichia coli* which catalyze the synthesis of RNA under certain conditions. The system (enzyme) works under the magnesium ions. The substrates needed for synthesis are nucleoside diphosphates—*adenosine diphosphate* (ADP), *uridine diphosphate* (UDP) ; *guanine* diphosphate (GDP) and cytosine diphosphate (CDP) ; but all the four substrates need not to be present at the same time. However, there is a quantitative relation between the amount of nucleoside diphosphate and the amount of base in the resultant RNA polymer.

The primer DNA, when it is used with the magnesium or manganese ions, all the four ribonucleotide triphosphate are necessary to form RNA. Infact, if any one of them is missing, synthesis is inhibiting. The reaction for RNA synthesis is just similar to that for DNA synthesis. The reaction takes place as follows.



$$n\,(ATP+UTP+GTP+CTP \xrightarrow[Mg^{++}]{\text{Primer DNA} + \text{enzyme.}} \begin{Bmatrix} AMP \\ UMP \\ GMP \\ CMP \end{Bmatrix} + 4nPP$$

ATP=adenosine triphosphate	AMP=adenosine monophosphate
UTP=uridine triphosphate	UMP=uridine ,,
GTP=guanasine triphosphate	GMP=guanasine ,,
CTP=cytidine triphosphate	CMP=cytidine ,,

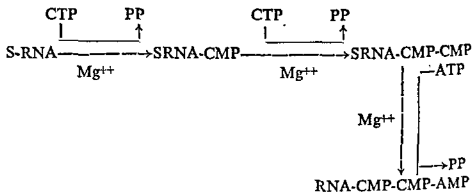
Primer DNA obviously serves as a template for RNA synthesis, because the order of the bases in RNA is complementary to that in the DNA. With ribonucleotide polymerase in vitro, both stands of primer DNA are copied while in the presence of same polymerase in vivo only one strand is copied. Investigations on RNA synthesis in RNA containing viruses have suggested that there may be an RNA-copying enzyme in these individuals. This theory of RNA production has been put forward by Weiss Hurwittz and Stevens (1959) which has been supported by many scientists later on. Ribonucleotide phosphorylase enzyme has the property of linking together to different ribonucleotides into an RNA chain, even in the absence of primer RNA. In vivo, Strickberger (1966) has demonstrated that the functions of ribonucleotide phosphorylase is not to synthesize the RNA molecule but breaking down RNA molecule.

The messenger RNA (m-RNA) is synthesized in *E. coli*, utilizing a DNA primer. This RNA is of nuclear origin and conveys genetic information from DNA in the nucleus to the ribosome in the cytoplasm in the protein synthesis. The another RNA is transfer RNA (t-RNA). It appears in the cytoplasm but probably it originates in the nucleus. In bacteria and in *E. coli*, transfer RNA consist 10 to 20% of the total cellular RNA. The ribosomal RNA comprises up to 80% of the cellular RNA and it can be identified and recognized by its sedimentation rate.

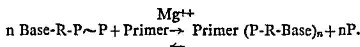
The latest finding on the relationships between RNA synthesis and the behaviour of lampbrush and polytene chromosomes support the conclusions of a DNA-dependent system. In general DNA synthesis is restricted to premitotic periods during interphase. However, to this time, it is not correct to generalise it, as it differs in different plants and animals.

Synthesis of the "free" Nucleotide (Coenzymes)—In addition of the nucleic acids proper, all cells contain a number of "free"

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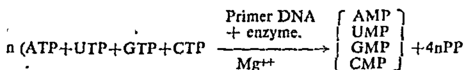


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Synthesis of the "free" Nucleotide (Coenzymes)—In addition of the nucleic acids proper, all cells contain a number of "free"

nucleotides. These *nucleotides*, however, includes the mono, -di-, *and triphosphates of adenosine*, guanosine. uridine, cytidine, and thymine. Although *certain* or *all* these compounds are intermediate in the *synthesis* or *degradation* of nucleic acids. However, many of *these compounds* and as *well* as *the* nucleotides containing bases which do not *occur* in the nucleic *acid* such as hypoxanthine, flavin, nicontinamide, *pantetheine*, fucntion as coenzyme *in* a *vide variety* of reactions. The free nucleotides may be synthesized as follows.

Mononucleotides—The synthesis and *degradation of the* purine and *pyrimidine* nucleotide coenzymes may be assumed to *proceed along the path* already discribed for the nucleotide constituents *of the nucleic acids*. However, an alternate pathway involved *reactions of* the *type* of phosphorylases, pyrophosphorylases, and kinases, by *means of which the* requisite nucleotide may be constructed stepwise from the free bases.

Dinucleotides

(a) **Nicotinamide Coenzymes**—The formation of dinucleotides is a much *complex* process. The synthesis of nicotinate has been *made from tryptophan*. This vitamin is provided by the diet. The *possible* of NAD formation may occurs as follows.

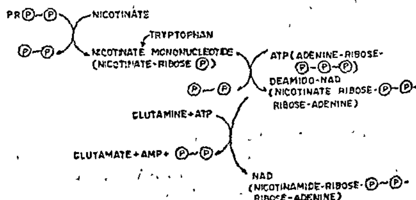


Fig. 145. Nicotinamide Coenzyme.

The phosphorylation of NAD by ATP and a kinase results in the formation of NADP. The NADP is reconverted to NAD by a phosphatase.

(b) **Flavin Coenzyme**—Although the carbohydrate moiety of *riboflavin is ribitol*, a sugar alcohal, the vitamin is usually considered a nucleoside, and its 5′-phosphate a nucleotide (flavin

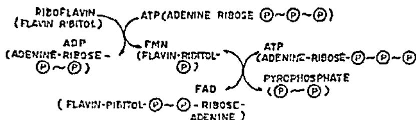


Fig. 146. Flavin coenzyme.

mononucleotide, FMN). The nucleotide is formed under the influence of a kinase, and is converted to flavin-adenine dinucleotide (FAD) by which occurs in the synthesis of deamido-NAD. FAD is split at the pyrophosphate linkage by the same enzyme that attacks NAD and NADP. FMN is hydrolytically dephosphorylated by a phosphotase.

(c) Coenzyme—This coenzyme is synthesized in much the same manner as other coenzyme. Pantothenate is first of all phosphorylated, then condenses with cysteine, forming a phosphopseudopeptide which is decarboxylated to yield phosphopantetheine.

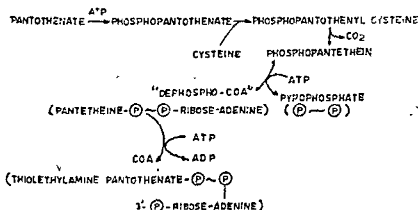


Fig. 147. Coenzyme A.

The phosphopantetheine acquires an adenylate component in a pyrophosphorylase reaction. CoA is finally produced by another kinase reaction.

Coenzyme forms of vitamin B_{12}—The compounds of this type do not fit readily into the preceding classification, since they are no less legitimate mononucleotide then FMN, they sometimes also

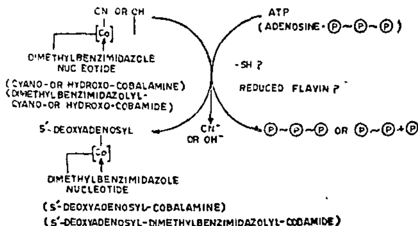


Fig. 148. Coenzyme of Vitamin B_{12}

contain a nucleoside moiety. An example follows the conversion of cyano or hydroxoforms of vitamin B_{12} to a coenzyme form.

SUMMARY

The synthesis of the nucleic acids involves the due consideration of the synthesis of purine and pyramidine ring systems, the orign of sugar and polynucleotide. Liver is the primary source of purine in mammal tissue. It is synthesized there. The detail mechanism has been dealt in the account. The biosynthesis pathway of the pyrimidine has been established mainly on the work with microorganisms using the mutant technique. Several reactions occur stepwise in the synthesis of pyrimidine ring. The biosynthesis of cytosine derivative take place by the conversion of uracil to cytosine under the influence of enzyme CTP synthetase The synthesis of DNA occurs the process generally known as replication. This however occurs in the presence of enzyme polymerase and Mg^{++}. DNA duplication, is however, inhibited by acridine drugs. For replication, all the four types of bases are needed. The synthesis of RNA occurs through RNA transcription. The process of RNA synthesis is bit difficult in relation to DNA synthesis. The recent evidences suggeste that all of the RNA of the animal cell is synthesized in the nucleus. Four important bases are also needful but here uracil replaces the thymine present in DNA.

The synthesis of "free" nucleotides, *e.g.* mononucleotid, dinucleotides (nicotinamide, flavin coenzyme, coenzyne A and vitamin B_{12}, have been discussed in some detail. Mostly they occur through the process phosphorylation.

21

EFFECT OF RADIATIONS ON BIOPLASM
OR
RADIATION IN THE CELL ENVIRONMENT

Even a layman with little elementary knowledge of biology knows that life is ultimately dependent on Sun's radient energy because animals are dependent on plants and plants, eventually are dependent on photosynthesis, a process occuring merely on the mercy of light. In additon to this vital role of light in the living economy, a persistent environment of various radiations in which the organisms live produce innumerable adaptative effects. On the basis of broad out line, the radiation may be of three types.

(i) Natural radiation.

(ii) Radioactive radiation.

(iii) Ionizing radiation.

The natural radiation which usually affect the cell of unicellular or multicellular organisms include in addition to sun's spectrum, radio-waves from the sun and stars, the ionising radiations from the breakdown of the radioactive elements in the earth's crust and the cosmic rays coming from the sun and from the depths of space. Sunlight is the major source of radiations on earth. Its spectrum includes ultraviolet radiation, visible light infraved rays and radio-waves. Kiepenheuer (1959), Menzel (1959) and Robison (1966) from the studies on visible and infrared parts of the spectrum have demonstrated that sun is a body with a surface temperature of 6000° absolute. Kiepenheuer (1959) showed that the ultraviolet radiations are absorbed by oxygen high in the atmosphere, forming a layer of ozone. The ozone in turn absorbs somewhat longer ultraviolet rays thus reforming oxygen (O_3). This ozone layer is called "the ozone umbrella" and it exists about 25 to 30 miles high in the atmosphere and is apparently the agent which removes the ultraviolet radiation of very short wavelengths.

Radioactive emanations or rays, as found by Rutherford in 1902, consist of three components.

1 Gamma rays—They carry no charge and are non material. They are electromagnetic, penetrating waves of very short wave length with a velocity of light and weak ionising effect and are like *X-rays*.

2. Alpha rays—These are positively charged particles with mass=4 and charge=+2 and, therefore are Helium nuclei. Their velocity is 1/10th of light with high kinetic energy and high power of ionization but less penetrating due to high mass.

3. Beta rays—They are negatively charged particles with mass 1/1850 of hydrogen atom and charge of—1 and therefore are identical with *electrons and velocity equal to that of light with small kinetic* energy hence less ionisation power and more penetrating due to small mass and higher velocity.

Cosmic rays, on entering the earth's atmosphere consists of charged particles, mainly protons(+vely charged hydrogen nuclei), and in addition some heavier particles. After these particles collide with the atmospheric gases, the cosmic rays also incorporate the uncharged particles (neutrons), gamma rays are charged particles others than protons such as mesons (positively or negatively charged particles intermediate in mass between protons and electrons) and positive and negative electrons (korff, 1957 ; Ginsburg and Razoryonil, 1959 ; Anderson, 1960 ; Hafner, 1964 ; Bowyer *et al*, 1965 ; Burbridge, 1966). Several cosmic rays strike the bodies of living organisms every second and even the organisms which are not affected by visible and ultraviolet radiations cannot escape the cosmic rays. But because of the difficulty of screening them out, the effects of cosmic rays on life have not yet been fully investigated.

EFFECTS OF RADIATION ON CELL

(1) Photodynamic sensitization of cells—Photodynamic action is *defined as that type of photosensitization which occurs only in the presence* of oxygen and produces lethal effects on the cells. In this process proteins of the cell are slectively oxidized. The aromatic amino acid residues in the proteins are most susceptible as shown by *Blum (1964). Mayor and Melnick (19*6 found that

light produces photochemical or photobiological reactions. Raab (1898) found that protozoans were killed when exposed to strong visible light in the presence of dilute solutions of some fluorescent dyes, but that they were inaffected in the dark in the same solutions. On the other hand, even intense visible light had no effect in the absence of the dyes. Dyes of similar concentration illuminated in the absence of paramecia are not injurious to paramecia subsequently immersed in them (Blum, 1964 , Spikes and Glad, 1964 ; Spikes 1968). Protozoans, such as paramecia, possessing no pigment, are translucent and appear white because the particles in their cytoplasm pass or scatter all wave lengths of visible light. As they do not absorb light,so remain unaffected even by intense visible light. However, when a dye which absorbs light, such as erythrosin, is present in the medium, it becomes absorbed to the surface of the paramecia, and the energy of the light absorbed by the dye is transferred to the cytoplasm, resulting in injury or death. Such a dye is called a **photosensitizer** and its action is **Photodynamic** if molecular oxygen is required for expression of the effect (Spikes, 1968).

(ii) **Bunsen-Poscoe reciprocity Law**—If the light intensity and the time of exposure are varied in such a way that the product of the two is always the same, the photochemical effect remains the same. The principle is called the **Bunsen-Poscoe reciprocity Law**. The photodynamic effect obeys this law but fails if the intensity of light is extremely low so that the injury caused on one hand is simultaneously recovered on the other hand.

(iii) **Photodynamic action in Nature**—It occasionaly occurs in nature. The protozoan, *Blepharisma* if grown in the dark produces the red pigment which accumulates in the outer part of the protoplasm. Deeply pigmented cells are killed if exposed to intense visible light. The pigment extracted from coloured, *Blepharisma* cells also sensitizes colourless ones to visible light. (Giese ; 1946 ; Giese and Zenthen, 1949.)

Another case of natural photosensitization is that observed in the carotenoidless mutant strains of the bacterium, *Rhodopseudomonas spheroides*, which can carry on photosynthesis in the absence of oxygen but is killed by light when oxygen is present whereas the wild type parental form, which contains carotenoid, is not affected.

A. **Photodynamic effect on cells of higher animals**—It is occasionally found in the cell of higher animals, for instance, cells in

the unpigmented skin of cattle, eating certain weeds are sensitized by a dyestuff which is present in the leaves. The dye is absorbed during digestion and is carried by the blood to the cells of the skin. Exposure to the sun then results in a rash, an edimatous swelling or in extreme cases, lsion and death. In man a photodynamic effect is observed when abnormal metabolic reactions occassionally form products which sensitize the body to sunlight (Blum, 1964).

B. Response in Photoreceptor cells-Vision—All animals and plants show definite response to light. The response may appear to originate from the whole body, *e. g. Amoeba, Paramecium,* etc; or from some definite region of the cells, *e. g.* stigma in *Euglena* and other flagellates or from the photoreceptor as in the eyes of different degrees of complexity. Multicellular plants have also developed photoreceptors which usually consist of cells near the growing tip that pigment enabling them to absorb light which results in differertial auxin transport and differential growth at a distance from the receptors (Briggs, 1964).

By studying vision in detail, the cellular responses to light can be analysed. The visual process in its essential features is a cellular process and is much the same in eyes throughout the animal kingdom. The best study has been done on the vertebrate eye.

The vertebrate eye in its innerside is lined by the cells of the retina, which receives the light after it has passed through the cornea, aqueous humour, lens and vitreous humor. The corena and the lens are the refracting parts of the eye; the humor are the bathing media; the ratina is the seat of photochemical and excitatory processes. The ratina of many vertebrates is composed of two types of photoreceptive cells, the rods which are narrow and spindle shaped, and the cones which are cone shaped (Fig 149). Cones, mostly are present in the central part of mans eye ratina; both cones and rods are present in the rest of the ratina.

The cones are concerned with colour vision and the fovea is the centre of detailed form vision. Histologically all cones appear similar but physiologically several types of cones are known, each specialized for reception of light from a certain span of the spectrum. Although rods are incapable of distinguishing colours, they are sensitive to much lower intensities of light than the cones; therefore, they serve as photoreceptors in a much lower intensity range. At night a very dim light can be seen from the corner of

the eye because of its action on the rods which are more concentrated at the periphery of the ratina. If the head is turned so that the light falls directly on the fovea, the light seems to disappear because the cones are insensitive to such dim light.

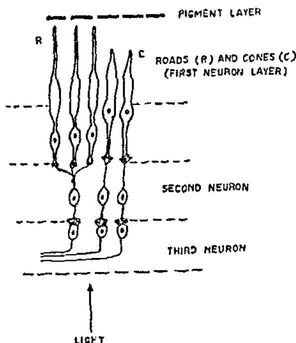


Fig. 149. Diagram showing the rods and cones in vertebrate ratina.

The ratina is bleached by light. When the dark adapted eye of an animal is briefly exposed to a brilliantly illuminated window and the eye is quickly removed and the retina separated and fixed in alum, it shows an image to the bright window.

The visual purple pigment or rhodopsin, may be extracted in the dark by rupturing with a detergent the rods of cells of a dark-adapted animal. Rhodopsin is a typical protein of large molecular weight with an absorption spectrum almost identical with the action spectrum for rod vision. The action spectrum is spoken to the relative efficiencies of different wave lengths for rod and cone vision in producing a biological effect. It is however readily bleached by light and regenerates to some extent in the dark.

Nature of the chemical reactions following absorption of light by rhodopsin in the rods of vertebrates will be clear from the following account. The spectacular recovery from night blindness observed in individual treated with vitamins A connected this

vitamins with vision (Dowling, 1966). Research on the problem indicated that dark adapted retains to possess little vitamins A, but light adapted retinas had an abundent supply when retinas from dark adapated animals were treated with strong fat solvents, a carotenoid called retinene (retinaldehyde) appeared in place of vitamin A. As this carotenoid disappeared during light adaptation, an equivalent amount of trans-vitamins A appear in the excised ratina. It has been actually found that vitamin A is the reduced form of relinene. During light adaptation the rhodopsin decom-

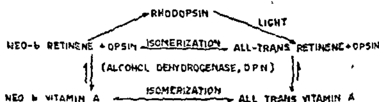


Fig. 150. The visual cycle cis and trans retinene.

poses to form trans-retinene and the protein opsin (Fig 150). The released trans-retenene than becomes reduced to trans-vitamin A (Wald, 1954). Retinene and Opsin obtained experimentally by action of light on retinas or retinal extracts do not recombine to form rhodopsin, nor do vitamin A and Opsin. From the experiments on optical isomers of retinene, it was discovered that although the cis-isomer combines with Opsin to form rhodopsin, the trans-isomer does not. It has been indicated that the isomerization from trans to cis-vitamin A normally occurs under the influence of isomerizing enzymes acting only on vitamin A, not retinene, consequently the reduction of retinine subsequent to isomerization are necessary for complection of the cycle. This was pointed out by Hubbard *et al*, 1965).

The cycle of the transformations involved in the breakdown and resynthesis of rhodopsin is called the visual cycle. The cycle is shown in the following figure 151.

Rushton (1959) has developed methods to measure the bleaching of rhodopsin in the intact eye and has shown that very *little* proportion (about 0 1 percent) of the rhodopsin is bleached even when the ratina is fully light adapted.

Just which of these chemical change if any, enables the rods to excite the neurons connected with them to carry massages to the

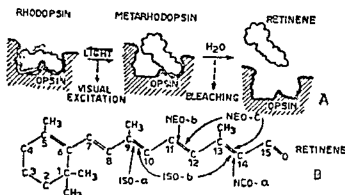


Fig. 151. A—The effect of light on vertebrate rhodopsins ; B—The structural formulae of retinene.

central nervous system is not yet known. The amount of energy required to stimulate the dark adapted eye of man is exceedingly small (only 5 to 14 quanta according to Hecht and coworkers (1942) for whole ratina). The pigment iodopsin, in the cones of the eye, has never been extracted free from rhodopsin and has been studied only in mixtures with the latter. There is evidence that vitamin A is also involved in visual response of the cones (Wald, 1964). In general, however, relatively little is known of the biochemistry of cone. vision at present, although the response of cones in intact eyes is under investigation (Rushton, 1959, 1962, 1964; Land, 1959, 1964; Wald and Brown, 1965; Mac Nichol 1966).

Carotenoid and a pigment resembling rhodopsia are also present in the cells of the eyes of various invertebrates, *e. g.* the squid, the blue crab, and the king crab. However, there is a little difference in the visual process of the animals, since when the squid's eye is exposed to light, vitamin A is not formed from retinine as pointed out by Wald 1954. Even the flagellates with eye spot, *e. g.* euglena, carotenoids are associated with responses to visible light, and the light absorbed most frequently by these substances is most effective in phototoxic responses.

C. Circadian Rhythms—Light has an immense effect on daily rhythms which occur in the life activities of unicellular plants and animals and in cells of multicellular organisms. Previously, it was thought that the natural alternating periods of day and night impose this rhythm, but if the rhythm is related to environmental periodicity in this simple causative way it should disappear when the plant or animal is placed in continuous light or darkness. But it has been

investigated by experiments that in some non-photosynthetic cells the rhythm usually persists in continuous darkness or continuous dimlight in some photosynthetic cells. The rhythm in continuous darkness or light is near about 24 hours, hence the name *circadian* (meaning about a day). The term have been used by Halberg and Howard, 1958; Aschoff 1963. Such an endogenus rhythm within cells suggests some mechanism which times the rhythm; hence the name biological clock have been suggested. Aschoff (1965) worked on biochemical and cellular rhythms in man. The circadian rhythm in unicellular dinoflagellate, *Gonyaulax polychedra* has been studied in detail by Sweeney and Hastings (1958); Hastings (1964); Hastings and Keyman (1965).

Further *Gonyaulax* is a photosynthetic cell which emits a brief flash of light when stimulated by agitation cultures grown in a daily cycle of day and night display rhythmically a greater luminescence upon agetation during the dark period than during the day. When such cultures are tranferred to a dark chamber,the rhythm continues, but its amplitude decreases progressively because the lack of nutrients, since photosynthesis ceases in the dark. Bright light permits photosynthesis but inhibits the rhythm, dimlight permits just enough photosynthesis to supply necessary nutrients to the cells but does not inhibits the rhythmic biolumenescent response which persists in dim light indefinitely without decrease in amplitude. Even cultures grown in bright light for a year, during which time the rhythm disappears, resume the rhythm when again grown in dim light. The period (the time taken from one peak to another) of the rhythm under constant dim illumination is somewhat less than 24 hours under low temperature, some what more under higher temperatures but always almost 24 hours.

If *Gonyaulax* is exposed to alternate periods of 7 hours of darkness and 7 hours of light, it becomes entran for a while to a new rhythm but when brought back to continuous dim illumination, the innate period reverts to 24 hours. From this experiment it appears that light, as a timer has a powerful effect but not a persistant one. This was indicated by Sweeney and Hasting, 1960, Hasting : 1964.

Some factors other than light, also play a part in circadian rhythms. For instance, Hasting (1960) noted that with increase or decrease of temperature there was always a little change in the innate period. The circadian rhythm is considered to be unaffected by the imput of information coming to the cell from the environment in the

form of the pressure (including even, osmotic pressure), mechanical disturbance ionizing radiations (eq. cosmic rays). The endogenous period depends upon the metabolic supply of energy, but it is unlikely that the period is determined by metabolic reactions in a simple manner, since the rate of most metabolic reactions is doubled by a rise of temperature 10C° (from physiology of metabolism) within the viable range, where as such a change in temperature has little effect on the endogenous period. So it is postulated by some that two coupled systems are involved, one of them light sensitive and temperature independent which serves as the pace setter and a second one temperature sensitive but light insensitive, phased by the first.

Many cellular activities in man and other mammals are also rhythmic. Although normally set to a diurnal rhythm by the day night cycle, they persist under constant conditions as circadian rhythms, being nearly, but not exactly of 24 hours periodicity. Man is a cave or in an underground isolated.

For example, continues to show a circadian temperature cycle and maxima in excretion of sodium, calcium and ketosteroids in the urine (Aschoff, 1965). There is evidence that numbers of white blood cells, the concentration of various hormones and certain nutrients in the blood, the synthesis of nucleic acids in some cells of the body and the susceptibility of the cells to drugs and toxins also show circadian rhythms (Halberg and Howard, 1958). Pizzarello *et al* (1964) claimed circadian rhythmicity in the cells of mice in response to ionising radiation.

Harker (1964) however pointed out that circadian rhythm in animals may take origin in certain cells of the body which then serve as pace setters for others. Strumwasser (1965) has noted the same observation in *Aplysia.* Further, these pace setter cells may evake the secretion of hormones in mammals which affect the *distant cells.* Secondly endogenous circadian rhythms of secretion of corticosteroid hormones from the adrenal cortex cells of mammals pace other biochemical activities in the body. The pineal gland in the brain is also given a significance as the major pace setter in vertebrates but it is thought to be dependent on other structures eg hypothalamus. Furthermore some of the activities of the pineal gland are under exogenous control by light (Wurtman and Axelrod, (1965).

(D) Photoperiodism—Photoperiodism is the growth response of cells in plants and animals to alternate periods of light and darkness and is correlated with the length of the light and dark periods.

Thus, some plants (long night plants) bloom only when exposed to a number of short days; others (short night plants) bloom only if exposed to a number of long days. This effect is the result of production of hormones in the tips of plants due to the response to stimulus of light which migrate downwards and result in growth or early maturity. These hormones do not pass through agar or cellophane. Variations in the intensity of the light (about threshold), such as are caused by clouding of the skies,do not affect the response which is regulated only by the number of hours of exposure. In the same way however the period of darkness is of equal value and its interruption even with a brief flash of light, alter the response. In other words definite alternate periods of light and darkness are required. Borthwick *et al* (1956) however, noted that germination of seeds and many other growth processes are also regulated by the same process. In this scientific period, many flowering cycles have been obtained by artificial control of light and dark periods from the plants with only one flowering cycle in nature. The hormones produced as a result of photoperiodic effect is considered to be a pigment called **phytochrome**. Though phytochrome is present in a exceedingly small amount about 1 part in 10 million, it has been extracted from the cells of tips of young albino plants and was shown to be a bluish compound consisting of a protein and another molecule attached to it. It has all the properties required for photoperiodic studies. Phytochrome is as widely distributed as chlorophyll and is as important to photoperiodism as chlorophyll is to photosynthesis (Hendricks 1964). Further more, the animal are also affected by photoperiodic phenomenon like plants. For example, many birds breed in the spring when days are getting larger, and precocious (early) breeding can be induced in winter by artificially lengthening the days, even when the temperature is below freezing. The converse pattern is found in some mammals that breed in the fall when the days are short and produce their youngs in the spring. In this case the *hypophysis* is activated by light stimulus which in turn stimulates growth of gonadial cells. Farner (1961) showed that photoperiodic control regulates breeding in many higher and lower forms of life as well as fat deposition, migration, and other behaviour patterns. In many insects reproduction can be controlled practically at will by manipulation of the photoperiod, although nutrition and temperature play a role as modifiers (Beck, 1963 ; Lees, 1968). The analysis of the cellular mechanisms involved in these responses is as yet rudimentary (Solberger, 1965).

(2) **Effect of ultraviolet light on cells**—It is well known fact that ultraviolet rays form an important part of invisible spectrum. Almost all human beings have experienced the effect of these rays *i. e.* the sun burn. The ultraviolet reactions in sunlight injure cells of the epidermis. The injured cells (prikle cell layer) liberated chemicals which diffuse out and cause a relaxation of the walls of blood vessels in the dermis, resulting in a reddening of the skin (erythema). If the injury is slight the red or the pink colour soon disappears, and in most individuals after a few hour's delay a small amount of pigment (melanin) develops. If the injury is more severe, the prickle cells may die, where as the layer is invaded by white cells and serum accumulates, causing a blister which later dries, and the skin peel's off as new epidermis takes the place of the old (Blum, 1955).

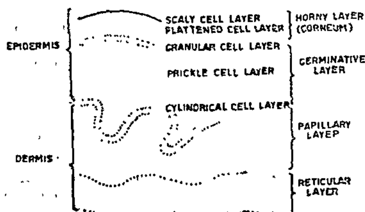


Fig. 152. Diagram of a section of human skin.

Moreover the shortest ultraviolet wave-lengths in sunlight which reach the surface of the earth and most effective in producing sunburn. Another harmful effect of the sun is eye burn or snow blindness. The superficial layers of cornea of the eye may be killed by the ultraviolet rays stricking the eye directly or after reflection from snow. Injuring resulting from ultraviolet radiation is superficial and leaves no permanent damage to the eye because after a few days, the injured cells become opaque and later the layer of the dead cells is shed. Blum (1955) however, noted although the ultraviolet radiation does not penetrate deeply but prolonged exposure induces superficial skin cancer in experimental animals such as rats and mice. Ever since Downes and Blunt (1877) discovered that ultraviolet radiation kills bacteria, all types of cell microbes, protozoans, egg of marine animals, algae, fungi and cells

in tissue culture. They have been killed even by ultraviolet radiations of wavelengths shorter than 310 nm (nm≈nanometer, or 10^{9} meter). Doses of ultraviolet radiation sufficient to prevent division of cells (*i. e.* to produce reproductive death) induce mutations in the surviving cells. Doses of the radiation milder than necessary for reproductive death retards the cell division, decreases respiration and affect most cellular functions especially synthetic processes (Giese, 1964).

Further, certain experiments have revealed that inhibition of DNA synthesis by ultraviolet radiation stop cell division. Larger doses progressively inhibit RNA synthesis and protein synthesis. This inhibition of synthetic processes probably underlies the various deleterious effects of ultraviolet radiation (Giese, 1964). Further according to Sinsheimer (1955) ultraviolet radiation is strongly and selectively absorbed by many organic chemicals and many compounds may be identified because of the characteristic and peculiar nature of the absorption spectra.

Photoreversal and photo-reactivation—Kelner (1949) found that visible light, *either* accompanying or *immediately following* ultraviolet radiation, will to a considerable extent, reverse the injurious effects of the latter, the phenomenon being called photo reversal or photoreactivation. Fungus spores, bacteria, animals and plant cells and viruses inactivated by ultraviolet radiation are photoreactivated by treatment with visible light (Jagger, 1958, 1960). Furthermore Vander Leun investigated that even erythema in the skin of man is cured by photoreactivation. Photoreactivation follows the dose-reduction principle by reducing and not completely abolishing the injurious effect. It should have in mind that only the blue end of the visible spectrum and the bordering long ultraviolet region are effective in photoreversal. Photoactivation appears to involve a photochemical reaction, which is than followed by a thermochemical reaction.

As has already been pointed out that DNA, RNA, and protein synthesis are sensitive to ultraviolet radiation and photoactivation. This fact can be proved by illustrating the following example. In the bacterium, *Hemophilus influenzae*, some strains are streptomycin resistant while others are not. The sensitive strain can easily be transformed into a streptomycin-resistant strain by addition of DNA taken from the resistant strain. But DNA of the streptomycin resistant strain is altered when subjected to the ultraviolet radiation and apparently loses its capacity to transform

the streptomycin sensitive strain into streptomycin resistant form. If the altered DNA is illuminated with visible light before it is added to the streptomycin sensitive strain, there is no photoreversing effect in the DNA-altering process If, however, a crude extract from yeast or from some other organism that shows photoreactivation is added to the irradiation-altered transforming DNA before illumination, then photoreactivation is obtained. Yeast possess an enzyme (photoenzyme) which facilitates photoreactivation of the ultraviolet treated DNA from the streptomycin resistant Hemophilus. It is derived from this fact that the enzyme of yeast which facilitates pnotoreversal combines only with the ultraviolet treated DNA, and DNA-enzyme complex absorbs visible light which photoreverses the ultraviolet injury of the DNA. Ultraviolet radiation, then, alters the DNA molecule, making possible combination with the yeast photoenzyme while the unradiated DNA does not combine with the enzyme. Further Rupert (1964) described that an extraneous DNA irradiated with ultraviolet, acts as a competitive poison in the system, uniting with the yeast enzyme and interfering with photoreactivation of the Hemophilus transforming DNA. In this way irradiation alters some bond, presumably in every DNA molecule, which make it possible of uniting with the photoreversing enzyme in yeast.

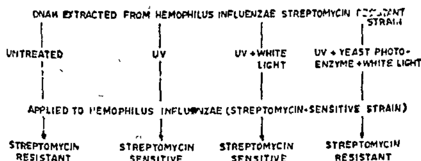


Fig. 153. Diagram showing the photoreactivation of the transforming principle of *Hemohpilus.*

(A) DNA^{H} +E ———→ DNA^{H}+E (no combination).

(B) DNA^{H} +UV———→ (DNA^{H}) UV (alteration),

(C) (DNA^{H}) UV+E→ (DNA^{H}) UV·E (enzyme complex).

(D) (DNA^{H}) UV.E+visible light→DNA^{H}+E.

The ultraviolet radiation in sun-light injure small organisms

or cells and induce mutations, but the accompanying visible light greatly reduces its injurious action

Dark recovery—**Hollaender and Claus** (.936) described the Liquid Holding Recovery. This discovery have also been named as dark recovery as it occurs in the dark. When *E. coli* culture had been exposed to ultraviolet radiation in one case immediately plated on nutrient agar and another case allowed to remain for a number of hours in buffered salt solution in the dark before plating. It has been noted the number of colonies in the latter case were much more numerous than the former. This is due to dark recovery. It is further noted that the different mutants of *E. coli* show different degrees of recovery from ultraviolet radiation. **Setlow and Carrier** (1964) demonstrated that during dark repair dimers (conjugated compounds in double molecules) produced by ultraviolet radiation were removed. This repair mechanism, due to the dark recovery has been named the cut and patch mechanism. Dark recovery follows injuries by certain agent other than ultraviolet radiation.

(3) Effects of ionizing radiations on cell—The ionizing radiations are the radioactive radiations which include—alpha or beta particles, fast or slow neutrons, gamma rays,and hard or soft X-rays. However, the brief exposure of the cells of the skin to these rays leads to a reddeming or erythema, which is followed by slight tanning. Furthermore such a brief exposures are used in the treatment of various skin diseases. More prolonged exposure, however, leads to skin injuries which are quite different from those caused by ultraviolet light. It has been noted that many ionizing radiations (such as hard X-rays) readily penetrate through a considerable thickness of the tissue. Thus the effect of ionising radiation is not confined to the surface, and thus the deep seated cell also injured. Certain cells are especially sensitive to the ionizing radiations (such as lymphocytes and proliferating cells). The cells of the germinative layer are more readily destroyed than other cells. In the same way the blood forming cells are selectively killed, and thus there is a change in the number and the proportion of the various types of cells. Moreover the gametogenetic cells, are selectively affected by ionizing radiation. As a result mutations are induced in gametes formed, therefore these radiations are the choice for genetic experiments. The greater doses than mutagenic ones inhibit gametogenesis completely, thereby sterilizing the organisms. This has been pointed out by Alexander (1965). Further Sparrow and Miksche, (1961);

Kaplan and Moses (1964) noted that the cells those contain th *greater amount of DNA, show greater sensitiveness to ionizin* radiation. Further the nucleus of the cell is more sensitive to th ionizing radiations than the cytoplasm. It has been noted tha when a wasp egg with asymmetrically placed are inradiated, single alpha particle delevered to the nucleus prevents division whil the million particles are required to do the same in the cytoplasm.

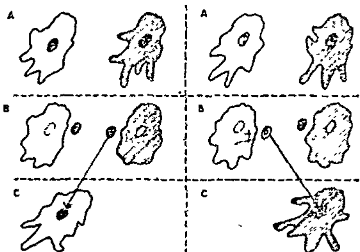


Fig. 154. Relative sensitivity of nucleus and cytoplasm to ionizing radiation to the nucleus of Amoebae.

The doses which produce reproductive death do not necessaril stop DNA synthesis. Further the RNA, and protein synthesis i also not much affected by a dose that inhibits cell division (repro ductive death).

BIOLOGICAL EFFECTS OF THE ATOMIC BOMB

*An atomic bomb explosion, which is at present, very commo*n in War and also for test, develops in its centre a temperature o about 1,000,000°C. The most destruction by atomic bomb are du to the result of its extreme heat, the ultraviolet flash, shock wave and ionizing radiations. The temperature of an object arround 400 yards from the explosion of the bomb, raised about 50°C. The ultraviolet flash burn may be severe. Therefore the surface cells are readily damaged whether they are micro-organisms or cells in the surface of the organism. The different shock waves rip the capillaries of the sense organs and lungs and thus cause hemorrhage throughout the entire digestive system. The ionizing radiation consist of about 15 percent neutrons and about 15 percent alpha

and beta particles and the reminder consists of short gamma rays Different cells such as protozoan and bacteria and body cells of an animal or plants, are injured in proportion to their sensitive to these radiation.

A characteristic set of symptoms in a person injured by ionizing radiations is nausea accompanied by vomiting, prostraction and, after a latent period, fever, bloodly diarrhoea, loss of hair and appearence of purple hemorrhagic spots in the skin. Various skin defect appear in areas where the germinative epithelium has been killed by the radiation. If, the infection so created, is prevented and the wounds heal, great sausage like growths of connective tissue known as Keloids develop over severly burnt areas of skin. The connective tissue of keloids may contract enough to make it possible for the victim to use hands, legs and other parts affected. Delayed effects includes mutations in the progeny of the exposed organisms as pointed out by **Los Alamos Lab** (1950).

Effect on cellular mechanism—Not only this but radiation influences many biological mechanisms. For example, aerobic phosphorylation shows impairment within 30 minutes after irradiation by 50 roentgens. The most conspicuous demage resulting from irradiation occurs in the chromosomes. The chromosomes undergo breaking in some areas, whereas in other they may adhere to one another and thus interfere with the normal process of mitosis. The influence of radiation on the chromosomes accounts for the well-documented instances of genetic mutations that so often result.

Irradiation causes cytoplasmic swelling and other changes in the mitochondria. Along with these alteration, inhibition of respiration and of phosphorylation, an increase in ATP, and an altered lipid metabolism have been reported. Certain more irradiation causes cytoplasmic swelling and the development of giant cells. This however, is not to be interpreted as a stimulus to growth. The giant cell represent impairment of division. Thus the irradiated cell, instead of dividing, simply continues to enlarge and ultimately dies.

Further, since irradiation impairs so many intracellular activities, it is not surprising that large doses decrease cell mobility. Thus sperm become immobile as well as infertile. Also, phagocytosis has been reported to be impaired following irradiation. Radiation also decreases the permeability of cell membranes By such effect, various substances are not transported by the cell membrane as readily as they are before exposure.

Mechanism of radiation effect—Radiation disrupts most cellular activities. But significantly, unless massive doses are used which prove immediately fatal, the alteration so far studied, for the most part, occur only after some measurable delay. The finding can now be explained. Large, immediately fatal doses of radiation produce such molecular chaos that vital intracellular activities are brought to an immediate end. Smaller doses, however, of about one-tenth of fatal dose, act primarily on the chromosomes, on the DNA-RNA mechanism. Very small doses will result in the chromosomal breaks and abnormal recombinations. The cell may still be able to undergo replication but the new cells will show various mutations.

Somewhat larger doses may so disrupt the genetic material that the cell cannot divide, yet all other activities such as protein synthesis continue to function. These cells become very large and ultimately die of without reproducing.

Moreover, the still larger doses render the genetic material useless. Cells so exposed carry out their metabolic processes untill the messenger RNA that was present before irridiation is used up. Now, because no more RNA can be produced, the machinary grind to a stop. This effect of radiation on the DNA-RNA molecule accounts for the characteristic delay in alteration observed in various metabolic functions. The genetic changes are the primary result of radiation, the metabolic changes are secondary. As radiation passes through the tissues, it colloides with atoms, thus producing ions which causes biological effects.

Lethal levels of radiation—Radiation in sufficient quantity is lethal. Exposure to a quantity of radiation in excess is 400 rods to the entire body will prove fatal to over half of the individual exposed. The sequence of events preceding death varies somewhat in different individuals but it usually involves nausea, diarrhoea, fever, hemorrhage and delirium. If the victim lingers a weak or more, profound anemia and a very low white blood cell count usually develop. And should he somehow survive for longer periods there may then develop leukemia and other forms of cancer. Thus if such a large quantity of radiation does not prove immediately fatal, it will undoubtedly produce serious biological effects in time.

SUMMARY

The universe speaks in many languages ranging from long wave length of radio-frequencies to the ultra short waves of X-rays. Each language conveys different information and is recorded by a special instrument "tuned"

to its frequencies. Out of so many languages spoke by the universe, one is the radiation and its effect on living beings.

Visible light, which is one type of radiation, is used by plant cells for photosynthesis. Light is also used by certain organisms to regulate cycles or to initiate various processes. But the radiation of visible light (natural radiation) can also have a deleterious influence on cellular activities by different ways. This is also true to great extent with other type of radiations ranging from ultraviolet light to gamma radiations. It has been pointed out that the radiations which have wavelength more than 2500A° are injurious to the cell. The eye burn or or snow blindness injury is also caused due to ultraviolet radiation on eye cell.

The cosmic rays imperil space travellers by creating undesirable genetic mutation in them. The invisible electromagnetic storms caused by sun radiations affect human life most profoundly. Study of the influence of electromagnetic radiation on life is an important problem. Any range of electromagnetic radiations affect the evolution and vital activities of organisms.

The effect of ionizing radiations is in two ways: (1) effect on germinal tissue and its inheritance in succeeding generations and (2) it affects the somatic tissue. The ionizing radiations result in somatic or germinal mutation. Ionizing radiations consist of 15 percent neutron, 15 percent α and β particles and remainder consists of short rays. Certain cells have been noted very sensitive to the ionizing radiation.

The radioactive radiations mainly consist of three types, which are caused by the three different types of rays, *i.e.* α-rays, β-ra[illegible]
body to great extent [illegible]
tissues, it, however, [illegible]
ions. In the process of ionization, chemical changes occur which cause biological effects such as burn, killed cells or altered mechanism of heredity.

In this way, the high energy radiation is very much injurious because it penetrates the body which produces [illegible]
ior [illegible]
is [illegible]
in [illegible]
chromosomes.

22

CHROMOSOMAL ABERRATION

The mutation is generally defined as a genetic change in an organism and this change being expressed as a modification of form and function. A change in the gene which is passed from one generation to the next is a permanent change in the genome of an organism. Specifically there are two types of mutations. The first being the **gene mutation or point mutation.** This can be interpreted in the term of physical or chemical change in the organisation of the gene at the molecular level. The second type of mutation is characterised as a gross morphological change in the structure of the chromosome. This gross morphological change always takes place due to the breaking of the chromosome into segments, and the segments might become reattached in a new arrangement. This happens in an orderly manner when crossing over take place. But however, occasionally chromosome segments undergo irregular breakage and rearrangements. These gross change in the chromosomes is usually termed as **chromosomal aberration or chromosomal rearrangement.*** Chromosomal aberrations have been found in a variety of plants and animals and thus have influenced the evolution in a number of organisms. In brief this chapter consists the aberration and their behaviour and fate in the organism in which they occur. There are two types of chromosomal aberrations. The first is **spontaneous** and the second is **induced aberration.**

SPONTANEOUS ABERRATION

The **spontaneous chromosomal aberrations** are normally naturally occuring rearrangements of the chromosomes. The origin of this type of rearrangement is not known. Several probable causes have been suggested but none has been substantiated. Cosmic radiations, nutritional insufficiences, and environmental conditions (temperature, etc.) may contribute to the production of these abnormalities. In some of the cases it has been demonstrated that a gene or genes promote the breakage of chromosomes as in the **dissociator-activator**

* For details consult author's "Text Book of Genetics".

system of corn. This peculiar genetic system was discovered during the study of bridge-breakage-fusion cycle of chromosome 9 (nine) in corn. A series of events beginning with the chromosome breakage and preceeding to the formation of a chromatid bridge in anaphase of meiosis occur in bridge breakage-fusion cycle. Spontaneous aberrations are very infrequent and in most organsims they are rare. The four principal types of spontaneous aberrations have been noted: They can be named as deficiencies, duplications, translocations, and inversions. Most of them involve one or more break in the chromosome Deficiencies and duplication involve loss or addition of genes to a normal gene complement, hence the carriers of these chromosomal aberrations are as a rule distinguishable from normal representatives of the species to which they belong by their appearance. Translocations and inversions change only the arrangement of the genes in the chromosomes, not the quality or quantity of the genes. For this reason they are sometimes referred to as chromosomal rearrangement. Individuals carrying such rearrangemets should be phenotypically entirely normal unless the relations of a gene or genes to adjacent genes effect the phenotypic expresion.

1. Deficiencies—A deficiency is caused from the loss of a part of chromosome. There may be two types of deficiencies. The first may be the terminal one, in which the loss affects the end of chromosome. It is produced by a single break near the end of the chromosome. By this break a small acentric piece is formed. The second deficiency may be interstitial and it affects the body of the chromosome. This is produced by the two breaks in an acentric region of the chromosomes thus giving rise to an acentric fragment. The chromosome lacking a segment is deficient for the genes present in the segment and this loss of the genes may have a phenotypic (observable) effect upon the organism. After sometime the fragment or fragments are lost due to the lack of a centromere. Moreover it is unstable. During the first mitotic or meiotic division the fragments move to one pole or the other following its production or it may remain in the cytoplasm, eventually to disintegrate. If the piece is included in the daughter nucleus, it is lost from the cell in the later division. The chromosome parts existing in the daughter cells can often be recognized as more or-less rounded bodies. Such bodies usually called as micronuclei. It is sometimes utilized in quantitative determination of chromosomal demage. A deficiency or micronucleus can vary in size ranging from a minute, submicroscopic segment to a major portion

of the chromosome. It has been noted that larger the loss or deficiency, the greater will be the genetic effect which will be very much injurious to the organism. If a very large part is lost, the effect may

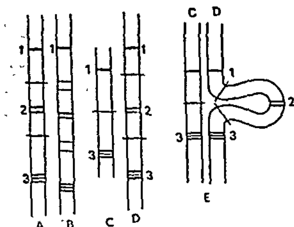


Fig. 155. Diagram showing the deletion in the salivary gland chromosome. A—normal chromosome ; B—middle segment (2) is deleted ; C—reconstituted chromosome after deletion ; D—normal homologous chromosome ; E—synapsis of two chromosomes in the loop formation.

be lethal to the cell. Furthermore, if the deficiency is sublethal, it may cause some functional or morphological changes in the organism. This is passed to the future generations as a gene mutation. In many cases the deficiency is so small that it has no effect upon the organism. Certain organisms are capable to withstand the loss of an entire chromosome without any significant demage. If the deficiency is occurring in only one of a pair of chromosome, it would be a cause of a heterozygous deficiency and would probably act like a recessive mutation.

2. **Duplications**—There may be two types of duplications. One type of duplication results from the addition of a segment of a chromosome to a chromosome. The segments may vary in size and therefore in genetic constitution. Another type results from the addition of a small centric piece of chromosome to a genome. Duplication by the addition of a segment to a chromosome depends upon breakage in the chromosome since a new chromosome segment can not fuse with a normal chromosome end. The duplications can be detected cytologically in a polytene chromosome as an extra band or bands. A duplication-deficiency situation is produced

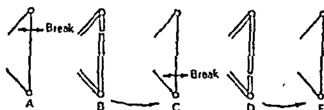


Fig. 156. Bridge-breakage fusion cycle in corn. A—bridge at meiotic anaphase; B—fusion of sister chromatids after duplication; C—bridge at meiotic anaphase; D—fusion of sister chromatids; E—bride.

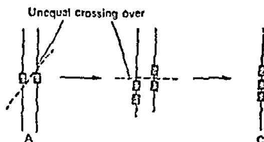


Fig. 157. Results of crossing over in the bar eye and bar-double eye. A—normal; B—bar; C—bar-double.

in a bridge-breakage fusion cycle (Fig. No. 156). In the anaphase bridge-break, one daughter nucleus has one extra segment of chromosome and thus a duplication of one or more genes, while the other daughter lacks that particular set of genes, at least in the heterozygous deficiency.

Types of duplications— The three basic types of segment duplications have been noted, *i. e.* tandon duplication, reverse tandon duplication, and displaced duplication. In tandon duplication the added segment has the same genetic order as the original segment and is adjacent to the original segment in the same chromosome. In reverse tandon duplication the segment is adjacent to the original segment in the same chromosome, but its genes are in the reverse order. In displaced duplication, a segment is inserted into different chromosome.

ABC ABC DEF

a

ABC CBA DEF

b

ABC DEF

LMN ABC OPQR

c

3. **Translocation**—First of all translocation was discovered by Bridges (1923) in *D. melanogaster*. Translocation is very normal phenomena which usually occurs in the chromosome. A translocation results from the transfer of a segment of a chromosome to a different part of the same chromosome or to a different chromosome. In the latter case the transfer may take place between homologous chromosomes or between nonhomologous chromosomes. If however, this exchange of segment occurs between the nonhomologous chromosomes, this translocation is termed as reciprocal and the subsequent meiotic behaviour of the chromosome become altered for a potential genetic effect. In the case of simple translocation a small segment of the chromosome is added to the end of the same chromosome or a different chromosome either a homologue or not. Simple translocation rarely occurs in the nature and it is due to the fact that normal chromosome end shows the inability to fuse with any other chromosome end. The third type of translocation is known as shift type. It involves the insertion of an interstitial piece of a chromosome into a different portion of a nonhomologous chromosome. Shift translocation requires a minimum of three breaks in the chromosome. It should be kept in mind that the reciprocal and shift translocations are the most common types of translocation.

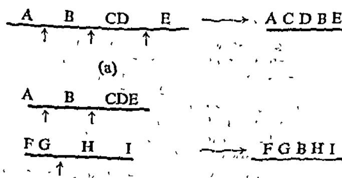


Fig. 158. Shift translocation
a—shift within a chromosome.
b—shift into a non-homologous chromosome.
(Vertical arrows indicate breaks).

4. **Inversion**—This is the most complex type of chromosomal aberration. The inversion is the relignment of an interstitial segment of a chromosome. The inversion requires two breaks within the chromosome and the reinsertion of the segment between the breaks in the direction opposite to its original position in the chromosome.

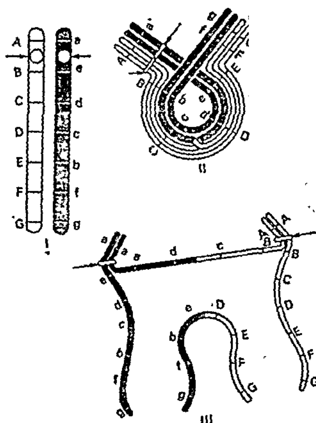


Fig. 159. Diagrams showing the crossing over in the heterozygote for a paracentric inversion. I—2 chromosomes which differ in paracentric inversion ; II—Diplotene state in meiosis showing chiasma inside the inverted part ; III=Anaphase of first meiotic division showing chromatid bridge and acentric fragment.

The linear order of the genes is thereby reversed in the particular section of the chromosome. This can be well represented by the example, if the arrangement of the genes on the chromosome were originally ABCDEFGHIJK, and an inversion occurred between D and H, the inverted chromosome would have a changed gene sequence as ABCDGEFHIJK. The difference in the length of the inversion chromosome would depend according to the location of the two breaks in the chromosome. There are two types of inversion, *i e* paracentric and pericentric. The inversion may be paracentric, if the inverted segment does not include the centromere. The inversion may

be pericentric, if it includes the centromere. This must be kept in mind that the homologous chromosomes with identical inversion undergo normal meiotic pairing and distribution during the meiosis. If only one homologous has undergone inversion, the normal meiotic pairing are disrupted, and the chromosomes take on a characteristic appearance. Inversions are well evident in the polytene dipteran chromosomes because of the clarity of specific segments as identified by their banding patterns. Moreover, the sequences of a heterozygous inversion are varied and mostly depend upon the position of the inversion, *i. e.* paracentic or pericentric, the presence and the number of chiasmata within the inversion, and the distribution of the meiotic products in the gametes. In the case of the paracentric inversion with crossing over, a bridge is produced in anaphase I of meiosis, and an acentric fragment is lost. The bridge may break at any point, thus giving rise to duplications and deficiencies in the mitotic products.In this way the fragments associated with the bridge has the effect of a deficiency and the size of the deficiency determines the reduction in fertility imposed. It means, a meiotic product or gamete lacking one or more genes due to the loss of a portion of the chromosome is likely to be nonviable and unfunctional and thus completely sterile.In the same way the products of heterogygous inversions, as well as the variations due to the chromatid exchanged during crossing over and to the number of chiasmata within the inversion, cause the change in the viability of the gametes.

With the simple inversion as shown above, a single chromosome however, may have two independent inversions of this type. More complex still is the case in which one inversion is included within another, or overlaps another in the same chromosome. Observations of inversions have added to the information on the phenomenon of crossing over. There is, however, further proof that crossing over occurs when four chromatids are present—and that it occurs between only two of the four chromatids at any one locus.

INDUCED ABERRATION

The main difference which lies between spontaneous and induced aberration is in the frequency with which they occur. The use of any physical or chemical agent simply to increase the frequency of chromosomal aberrations, but it does not create new types. Most of the agents inducing mutations also induce breakage in the chromosomes. Radiations have been longly utilized to cause mutations and chromosomal demage for experimental purposes. But there is only one point

which distinguished the mutation from chromosomal aberration, that the number of the mutations is directly proportional to the dose of ionizing radiation, but such a ratio does not hold good for chromosomal aberrations.

In 1927 Muller presented experimental data which established that the radiation could induce mutations in living organisms. Riley, Sex, and others, thereafter began to study the radiation-induced chromosomal aberrations. In this way various kinds of radiations those usually cause mutations, chromosomal aberrations and other cellular effects. The first radiation is the X-rays and gamma rays nonparticulate radiations which is known as a quanta of energy, producing a series of ionizations in the material exposed. Upon exposure to ionizing radiations, electrons are raised to higher level of energy and are ejected from the atom. Thus primary (initial ejection of electron) and secondary (energetic ejection of electron from adjacent atom) radiation can induce a change in the molecular organisation of protoplasm. The change may be expressed either in term of mutation or a break in a cl romosome, or an alteration in the physiological activity of the cell. The second type of radiation (particulate) ionising radiations include alpha particles, beta rays, protons and neutrons which are used as a tool for biological studies.

There is considerable variation in sensitivity to radiation at all levels of biological organizantion. Simple individuals such as bacteria virusus and protozoans are much more radioresistant than the more elaborate plants and animals. The most sensitive organism belong to the primate group, which include man. Dividing cells, mitotic and meiotic, are more sensitive than non dividing cells, expressing their sensivity as lethality, mutations or chromosomal aberrations, or changes in the cellular metabolism, such as the disruption of DNA synthesis or protein synthesis. From the stand point of a tendency to mutation or chromosomal breakage, late prophase and metaphase are more sensitive than other stages, and the synthetic stage of interphase are almost as sensitive. According to the quality of the radiation, the intensity, and the dose, a cell may be prevented from dividing either permanently.

Radiation-induced chromosomal aberration can be divided into two main categories. The first is the chromosome-type and second is chromatid-type. The difference between the two lies in

he time during the cell cycle at which the break occurs in the chro-
nosomes.

Chromosome-type—If the chromosome is irradicated before luplication, the aberration observed in the subsequent metaphase ınd anaphase are of the chromosome-type. Chromosome aberration nay be further classified as **terminal deletions, interstitial deletions,** ınd exchange. A teminal deletion is the result of a single break in he chromosome at the terminal portion and is therefore represented oy a rod-like fragment without a centromere, consisting of a two hromatid arms. Further the duplication process occurs in both he portions. If the chromosomes and the fragments do not undergo estitution (restore the normal morphology of the chromosome) with-n a certain period of time, the fragment becomes separated from the ıormal body of the chromosome and is eventually lost because of he lack of a centromere.

It should be kept in mind here that the size of the fragment olays an important role and a terminal deletion can cause the loss of

Fig. 160. Chromatid abberration.

deficiency of one or more genetic loci. Thus it is comparable to the deficiency type of spontaneous aberration. An interstitial deletion is the result of two breaks in the chromosome, which occur at the same side of the centromere. An interstitial deletion is also basically a deficiency type of aberration and it is the most common in many organisms.

Inversion can also be induced by radiation or other treatments. An induced inversion in the meiotic cell is detected in the same manner as a spontaneous inversion. The inversion can not be detected in the somatic cell.

Chromatid aberration—If the chromosome is effectively double at the time of irridiation, the aberrations observed during the ensuing division are of the chromatid-type The difference between the chromosome and chromatid aberrations are shown in the (figs. 160 and 161). There are three main types of chromatid aberrations, *i. e.* **chromatid deletion, isochromatid deletion** and **chromatid exchange.**

Fig. 161. Chromosome aberrations.

As the chromosome consists of two chromatids at the time of irradiation breakage is possible in either one chromatid or both chromatids. The broken end may undergo (1) **restitution**, which restores the chromosome to normal morphology ; (2) **displacement**, which leaves a terminal deletion or (3) **reunion** with the broken end of another chromatid in the vicinity. In the last instance a chromatid exchange takes places. Although only one chromatid may be broken, breakage of both chromatids in corresponding position is more likely. This situation, however, produces four broken ends which may recombine (by restitution or reunion) in several ways.

Exchange are the most complex chromatid aberrations. They are designated as **interchanges** according to origin. Most of them can also be classified as translocation. If the interchange produces four normal-looking chromatids by anaphase, it is asymmetrical and is difficult to detect unless the sizes of the two interchanging chromosomes differ.Further an **asymmetrical interchange** results in the formation of a dicentric chromatid, with an associated acentric fragment and two normal chromatids. This asymmetrical interchange is recognized easily during metaphase and anaphase. Two break within the chromosome may lead to symmetrical or asymmetrical interchange but in the latter event a chromatid ring, centric or acentric, and a fragment are produced.

Radiomimesis—With the discovery in 1947 that mustard gas and similar compounds could induce mutations and chromosomal aberration, there began a series of experiments with a variety of chemicals to induce specific changes at the level of gene and chromosome. The chemical nature of these compounds were known it was thought that they might provide a key to the exact nature of the mutation and chromosomal breakage. It is noted from most of the experiments that the basis for the chemical effects differ from that of the radiation effects. Still, chemical agents produce the same kinds of effects as that of X-rays produce and so the name **radiomimetic** agent has been given to them which simply denote that their effects mimic those of radiation. These effects include breakage of chromosome, stickness, and mitotic inhibition.

The effect of these chemicals and radiation do not coincide in time. Some radiomimetic agents are most effective in inter phase (the most sensitive stage of the cell), where as others are very sensitive during mitotic cycle. The most widely used radiomimetic agents are the mustards and their derivative such as diepoxide,purine pyrimidine

the effects of radiation on human cells in Vivo, bacause human is among one of the most radiosensitive of all organisms.

CYTOLOGICAL MAP

The correlated studies on cytology and genetics on chromosomal aberration have permitted construction of cytological map of chromosome. It shows the location of various genes in terms of the microscopically visible chromosome. Cytological maps of metaphase chromosome of *Drosophila melanogaster* and *D. pseudoobscura* have been made by Tan Anderson where as others succeeded in making such maps for some chromosomes in maize. The linear arrangements of genes shown by cytological maps and also by the linkage maps are invariably the same. The work on cytologic maps has therefore fully confirmed the theory of linear arrangements of the genes on chromosomes, originally put forward on the basis of the studies on recombination of trait in crosses. However, the relative distance of the genes from each other in cytological and linkage maps do not always correspond.

SUMMARY

The gross morphological change, that occur due to the breaking of the chromosome into segments which might become reunite (reattached) in a new arrrangement, is known as chromosomal aberration. These changes occur in an orderly manner. They may be of followiug types.

(A) Change in gene number—This may be of deficiency or deletion type. In this the loss of one or more genes occurs. The second is the duplication in which there is a addition of one or more genes as a result of which an organism carries the same genes repeatedly in its haploid chromosome complement.

(B) Change in gene arrangement—This may be translocation (exchange of parts between the two nonhomologous chromosomes as to form a new chromosome) or inversion (when the exchange occur within the chromosome).

These all changes occurs due to the change in the number or arrangement of gene loci within the chromosome. These all are included in the spontaneous

analogues, phenols, etc. Their effect is also influenced by many factors such as temperature, pH, metabolic inhibitors and oxygen concentration. It is interesting at this place to note that oxygen alone, in high concentration and at high pressures, can induce chromosome breakage, although it is generally considered to be a mutagenic or radiomimetic agent.

The mechanism, how the radiomimetic agent acts is not known in many cases. But it is well known fact that their action is not the same in all cases. Certain agents, such as mustard and related alkylating compounds, may react with biological molecules, such as DNA, forming esters with the phosphate group. Recent investigations with such agents as 5-bromouracil, 5 fluorodeoxyuri dine and hydroxylamine suggest means by which specific purine and pyrimidine bases of DNA can be disrupted and replaced to produce mutations or chromosomal breaks. It is interesting to note further that most of the radiomimetic agents have the localized effect upon the chromosome. Their effect is mostly found in the heterochromatic regions of the chromosomes rather than euchromatic regions.

The frequency of aberration, especially the exchange types, lead to some interesting interpretations when X-rays are used with particular type of radiomimetic agents. The breaks induced by most chemical agents seem to have the capacity to undergo reunion with the break induced by X-rays. On the other direction, when certain chemical agents are used together to treat cell, the break induced by one agent do not necessarily undergo reunion with the break induced by the others. The implication here is that the process of breakage differs for various agents, or the physical or chemical characters of the broken chromosomes differ, thus prohibiting reunion.

The importance of the radiation effects on the chromosome at present is very necessary to study because of the daily increased levels of radiation in the atmosphere and on the ground and their inharent danger to men.* Several studies on the problem have been conducted to determine the rate and effect of radiations on human population in the nature and in the laboratory, and it has been found that the same type of chromosomal aberrations are induced in them as in other living cells. As most of the work in this direction [illegible] done on human cells, grown in vitro, much remain to b[illegible] [illegible]lishing

* For detailed study see previous chapter on Bio[illegible]lasm".

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aberration. The chromosomal aberration may also be brought about by artificial method. These aberrations are known as induced aberrations. In 1927, Muller presented the result of so many induced aberration. Radiation-induced chromosomal aberration may be classified into two main categories. The first is the chromosomal type and the second is the chromatid-type. The difference between the two lies in the time during the cell cycle at which the break occurs in the chromosomes.

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CHROMOSOME NUMBER

Chromosome number* has significance in species group as well as in individual plants and animals. Chromosomes occur in sets and a chromosome set or karyotype is generally constant for all the somatic cells of an individual. Somatic cells routinely have chromosomes in pairs (2n), *i. e.* two of each kind. Mature germ cells, having undergone reduction division, normally have one member of each pair (n). Thus with the exception of sex differences, somatic doubling, and minor variations that occur in natural and experimental populations, all members of the specices of plants and animals have the same basic number of chromosomes. Chromosome number can be overemphaized as a creterion for species indentification. As may be expected with any biological phenomenon, there is, in addition, a significant incidence of variation from the diploid or haploid condition in some organisms or in a specific cell type. The term heteroploidy is used here in broadest term, *i. e.* all the variations in the number of chromosome found in the living world. *However, certain variations in the number of chromosomes* are not always associated with the phenotypic in the organism. Likewise, changes in the number of chromosomes may be reflected in phenotypic variations. It is easy to deduct such a variation and the genetists are provided with valuable tools for identifying the action of individual chromosomes. Some plants with increased chromosome number have proved valuable because of phenotypic changes which add to their morphological or physiological values. Several aspects of these variations will be presented here, though they are not all related in their origin or biological influence.

Classification of heteroploidy or chromosome changes are arbitrary and superficial because all the changes are interpreted only in the term of obvious additions or eliminations of chromosome parts (whole chromosomes, or *whole chromosome sets). So* the present classification system is merely a working tool.

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23

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ANEUPLOIDY

The term aneuploid refers to irregular chromosome sets, made up of a part of genomes. Organisms with chromosome numbers that are not exact multiple of the monoploid set are called aneuploid. The aneuploid always come into the field with either additional or lesser number of the chromosome relative to euploid arrangement. This phenomenon is termed as hypoploidy and hyperploidy. These both arise from an abnormal distribution of the chromosomes to the pole during anaphase of meiosis. In this case one daughter cell receives an extra chromosome (or chromosomes) and the other lacks a chromosome (or chromosomes). The aneuploidy always arises due to the "nondisjunction" of the chromosome during the cell division. The first critical study of aneuploid plant was made by Blakeslee and Belling (1924) on Jimson weed, *Datura stramonium* which normally has 12 pairs of chromosomes in the somatic cells. These investigations announced a discovery of mutant type having 25 numbers of chromosomes rather than 24 chromosomes. Out of the 12 pairs, one pair was found to have an extra number, at the meiotic metaphase stage. One extra number was present in addition to the irregular set and thus the condition was illustrated by the formula 2n+1. In the same way Bridges (1916) recognised the phenomena and utilized it in his classical study of exceptional individuals in *Drosophila*. He referred to the process as one of nondisjunction, *i. e.* the member of a pair apparently failed to disjoin, both passing into the same anaphase nucleus. The two complementary gametes (one n+1 and other n—1) will join with the normal gametes, will give individuals with 2n+1 or 2n—1. Such individuals are commonly referred as trisomics and monosomics respectively. The particular chromosome in question is represented in triplicate or singly.

Monosomic aneuploidy—Monosomics, rare in normal diploid organisms, are very likely of polyploid origin and may persist in polyploid individuals through several cell generations. In a cell with more than the diploid number af chromosomes, the loss of a single chromosome would be expressed to have little or no effect on viability. The monosomics have been found in species of wheat and tobacco identified as polyploids. The deleterious effect of the loss of a single chromosome from a diploid cells is evident upon observation of meiosis in a monosomic individual, *i. e.* normally diploid,

Since the remaining number of a bivalent no longer has a homologue with which to pair, it behaves as a univalent chromosome, moving to either pole of the cell during division. The gametes developing with a deficiency for this chromosome are usually nonviable.

Primary Trisomic aneuploidy—Trisomic individuals have been found in a number of species including *Drosophila*, Datura, and corn. The presence of a third homologue chromosome in a cell undergoing meiosis interferes with the pairing process. However, under mormal circumstances two homologous chromosomes pair over their entire length giving rise to a bivalent in the prophase and metaphase of the first meiotic division. Since only two homologues can pair at any one position, three homologues will assume several arrangements. A trivalent configuration results from an association of all three chromosomes. On the other hand, two of the chromosomes may join to produce a bivalent, leaving the third as a univalent. The longer the chromosomes, the more likely the formation of chiasmata and therefore of a trivalent. An example of a trisomic individual is triplo IV of *Drosophila*, in which chromosome IV is present three times. The phenotypes include narrow pointed wing, coarse bristles, and smooth eyes. The genetical ratio which appears in the offsprings of such trisomic individuals mated to normal individuals are consistent with the expected pattern of segregation and are different from the ratios obtained when two diploid individuals are mated. The scheme of crosses between normal and a typical individuals is given below. Here the normal ratio is 1 : 1 ;

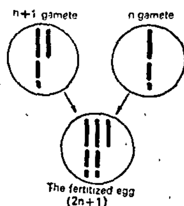


Fig. 162. The origin of trisomic.

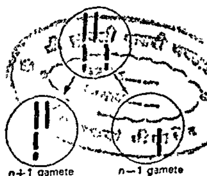


Fig. 163. Diagram showing nondisjunction.

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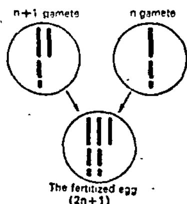


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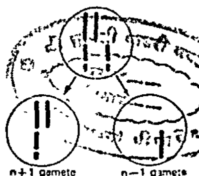


Fig. 163. Diagram showing nondisjunction.

the ratio in offspring of crosses between normal and typical individuals is 2 : 1 or 5 : 1 depending upon the viability of the gametes. All this can easily be designed as primary.

Normal A*a* × *aa*→

	a	*a*
A	A*a*	A*a*
a	*aa*	*aa*

→ 1 A : 1*a*.

Trisomic : A A*a* × *a a*→
(abnormal)

	a
2A	2 Aa
[2A*a*]	2 A*aa*
[1AA]	1 A*aa*
1*a*	1 *aa*

5 : 1 *a* if all the gametes survive or 2A : 1*a* if bracketed gametes do not survive.

Secondary Trisomic—It differs from the primary trisomic, that in an individual the third chromosome is not identical to the other two. These arise from the primary trisomics and in addition produce an altered phenotype when a bivalent and a univalent form acts abnormally. The centromere of the univalent chromosome undergoes middivision to produce isochromosomes. In this way the third member of the group in secondary trisomic is a chromosomes with two identical arms. In contrast to primary trisomic secondary trisomic results in a ring of 3 chromosomes during meiosis. For example *Datura* has 12 haploid number and however there are 24 possible secondary trisomics. Many of these have been observed, and each has a characteristically different phenotype. More complex trisomic are known in some *Datura*, such as tertiary trisomic in which translocation takes place between non homologous chromosomes and in which the pairing is disrupted in a fashion similar to that in other trisomic.

EUPLOIDY

Euploids have chromosome complements composed of whole sets (or genomes) of chromosomes. In contrast to the aneuploids, which differ in single chromosomes, euploids differ in multiples of n. Monoploid (n) carry one genome, *i. e.* one member of each kind or chromosome. The n number chromosomes is usual for gametes of diploid animals, but however, unusual for somatic cells. Monoploidy is very seldom observed in animals, but a few cases such as that of the male honey bee may be cited. Plants have a gametophyte stage in their cycle which is always characterized by the reduced chromosome number. In the higher monocotyledons and dicotyledons this stage is very brief and inconspicuous, but in some lower plants, it represents the major part of the life cycle. Monoploid plants, either occuring in the natural population or produced experimentally can be varified cytologically. These plants are usually frail in structure with small leaves, low viability, and high degree of sterility. The sterility is attributed by the irregularities which take place at the time of meiosis. Obviously, no pairing is possible because only one set of chromosomes is present. Therefore, if the meiotic process succeed at all, the dispersal of the chromsomes to the poles is irregular and the resulting gametes are highly variable. Because monoploides undergo no segregation and carry a single set of genes, they could be used experimentally to good advantage if they could be reproduced successfully. Howevery, the most common euploid is the diploid with one genome duplicated. This chromosome arrangement is nearly universal among animals and is very common among plants.

Although the origin of all spontaneous monoploids has not been determined, there are several ways in which they may arise. In plants an unfertilized egg may be stimulated to develop into an embryo by the growth of the pollen tube or by some other physical, and environmental stimulus. Some animal eggs and plant eggs can be stimulated to develop by exposure to electrical shocks, chemical treatment, or other experimental procedures.

POLYPLOIDY

Organisms with three or more genomes are **polyploids**. Polyploidy is not a rare condition in the plant world. About two third of all the grasses are polyploids. In contrast to the wide spread occurrence of polyploidy among plants, the condition is rare in animals. The important cause for the relative low number of

polyploidy in animals is their sex balance, which is much more delicate than that in plants. However, it has been noted that the addition of the chromosome above the diploid number give rise to intersexes which do not reproduce.

Origin of polyploid—Polyploids can arise in several ways. Spores or gametes may be produced by anamolous meiotic divisions in which there is no reduction in chromosome number. Somatic doubling of chromosomes occassionally occurs in both plants and animals and this leads to the formation of diploid gametes. In mosses and ferns it has been possible experimentally to regenerate gametophyte directly from the sporophytic tissue. Such gametophytes are of course, diploid and always produce diploid gametes. This process can be repeated several times, each time doubling the chromosome number. Finally the participation of more than two nuclei in fertilization, which regularly occurs in the initiation of endosperm tissue in seed plants can result in the addition of extra whole sets of chromosomes. It occasionally occurs in most plants and animals that, though irregularties in meiosis, non reduced

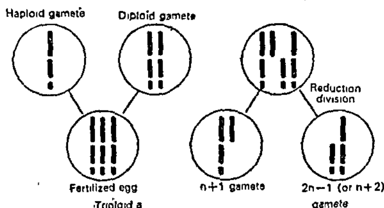


Fig. 164. The triploid.

spores and gametes are produced. As a result of the formation of diploid gametes it is possbible for either triploid or tetraploids to arise. It depends on whether one or both types are diploid. In most normal diploids, the frequency of formation of diploid gametes by such irregularties in meiosis is so low that the probability of obtaining tetraploids by chance meeting of two such gametes is practically zero. Though breakdown of the synchronism between chromosome multiplication and division of cytoplasm during meiosis

is possible for diploid cells to give rise to tetraploid cells. However, the descendent of such cells give rise to island or sector of tetraploid cells. In such sector happen to be the germ line, diploid gametes or spores are formed as a result of normal meiotic divisions which will give the tetraploid plants.

There are two major types of polyploids according to the origin of the chromosomes, *i. e.* **autopolyploidy** and **allopolyploidy.** These two may be distinguished on the basis of the source of chromosomes.

Autopolyploidy— A diploid species has its two similar genomes like AA, then an autotriploid become AAA and the autotertaploid AAAA. The latter would have its origin directly from the diploid by the union of two diploid gametes while the former could arise as an offspring of a tetraploid and diploid present by the union of an unreduced and reduced gametes, This is now believed that this process now very frequently occurs in the nature.

Autopolyploids are sometimes, in many respects are larger than their related diploids as a result of an increase in all sizes. But this criterion can not be used indiscriminately, however, for the increase in size depends upon the genotype of the diploids from which the autopolyploids arise. Other morphological characteristics of autopoloids as compared with the diploid include larger pollen grains and more chloroplasts. On the other hand, chromosome number higher than the tetraploid may result in plants, which are smaller and less viable than their diploid relative. These phenotypic variations are dependent upon the specific genetic makeup of the organisms in question and can only be considered as generalities to which there are exceptions.

Cytologically, autopolyploids are characterised and identified by the presence of multivalents formed at metaphase of meiosis I. In autotriploid the three homologous chromosomes pair with each other to give trivalents and in autotetraploids, quadrivalents would result. In this way the number is not constant for each cell and it depends on the degree of synapsis and chiasma formation, taking place in meiotic prophase. It is a fact that autotriploid are highly sterile because of the random segregation of the three chromosomes of each trivalent.

In varying degree of sterility in autotetraploids, however suggest that disharmonies of a genetic nature are more probably the basis of sterility than is irregular segregation, for segregation may

be reasonably regular yet high sterility is encounted. Stebbin (1949) has shown that Colchicine produced autotetraploid of the grass. Beasley (1940) in *Gossypium herbaceum* and Emset (1947) in cultivated lettuce pointed out that the autotetraploid are similar as regards chromosome relationships to the fertile types, are highly sterile. Ruttle, etl (1942) indicates that sterility may be partially co-related with disturbances in the latter stage of meiosis, but the fact that autotetraploids from different diploid varieties behave differently, points to a genetic basis of undermined nature.

Since in autotetraploid, each gene is represented four times, inheritance in such plants can be expected to be more complicated than the normal diploids. Further, the osmotic concentration of polyploids is also higher than that of the diploid plants. Vitamins, alkaloid and sugar contents of beet are also markedly increased. In some of the cases it has been found that plants which are annuals in the diploid stage become perennial when they are produced in the form of tetraploid. Tetraploidy also changes the season of blooming and fruiting Thus it can be concluded that autopolyploidy provides a method by which a type may become adopted to new and specially to less favourable conditions.

Allopolyploidy – In the case of allopolyploidy the genome constitution would be represented as AA B_1B_1 with the tetraploid having arisen by a doubling of the chromosome number of an F_1 hybrid between species A and species B_1. If the genome are sufficiently dissimilar to preclude any synapsis in the F_1 hybrid (AB_1), it is clear that the hybrid will be highly sterile because of irregular chromosome distribution during reduction division and however the doubling of chromosome number gives the allotetraploids AA B_1B_1. A classical example of an allotetraploid is *Raphanobrassica*, obtained by karpechenko from hybrid between raddish, *Raphanus sativus* (the diploid chromosome number, $2n=18$) and cabbage. *Brassica oleracea* ($2n=18$). However, raddish and cabbage cross with difficulty. The F_1 hybrid have 18 chromosomes, 9 of them contributed by the raddish and 9 by the cabbage parent. At meiosis, the raddish and cabbage chromosomes in the hybrid mostly fail to pair and in this way the meiotic division will be highly abnormal, and the spore usually degenerate, thus making the hybrid very nearly sterile—a typical case of sterility of an interspecific hybrid. However, in some cells the chromosome complement undergoes a doubling, and this leads to the formation of a few seeds, from which some second-generation hybrid can

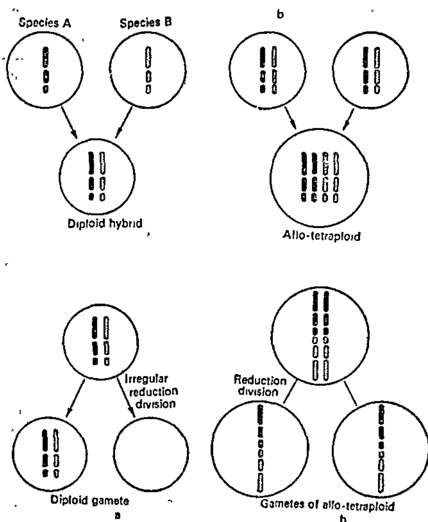


Fig. 163. The origin of allo-tetraploid.

be obtained. Most of the generations have 36 chromosomes, the sum of the chromosome numbers of the two parent species. Such tetraploid hybrid are remarkable for their giant size and even more so for their almost complete fertility and true breeding ; since their morphological characters are intermediate between raddish and cabbage, although they are infertile with both parent species, the name *Raphanobrassica* has been given to them. The chromosome behaviour at meiosis in diploid *Raphanobrassica* is entirely normal. The plant with 36 chromosomes form 18 bivalents and the embryo sacs and pollen grains carry 18 chromosomes. Nine of them representing the full cabbage complement and the other nine full raddish

complement. The new plants with 36 chromosomes produced as a result of fertilization. But however, the chromosomes, will have dissimilarities in the gene arrangements. This is the case of chromosomal sterility. At meiosis, raddish chromosomes do not find normal mates among the cabbage chromosomes and vice versa. On the other hand, the tetraploid carry even raddish and cabbage chromosome in duplicate and consequently every chromosome has a mate with a precisely similar gene arrangement, as the formation of the bivalents clearly shows.It should be kept in mind here, that alloploid hybrids are, however, by no means always fertile, nor do they always have normal meiosis.

Still more complex polyploids may occur and however their frequency in the population depends upon the several conditions, including homologies of chromosomes. Auto-allopolyploids have been found in several species of plants, most of them hexaploids. According to the designation, the formula would be AAAA BB, resulting from the union of an AA gametes with a B gametes, followed by a doubling of chromosome number. In this way an autoalloocto-ploids, AAAA BBBB could result from the union of two autotetraploids.

Heteroploidy in Tissue—Numerous examples of variations in germ tissue are known, but variations in somatic cells have also been observed. Certain tissues both in plants and animals have been found to contain more or less than the usual diploid number of chromosome with the differences being regular according to the nature of the tissue and the nature of the change in chromosome number. This somatic differences arise in several ways and are variously termed as somatic reduction, somatic segregation, and endopolyploidy.They appear in otherwise normal tissues as well as in abnormal tissues.

Somatic reduction—This process is well represented in the development of gall fly,*Miastor*.During the early stages of embryogenesis, certain chromosomes are lost from many of the cells. In *Sciara* and *Iceryo purchasi*, the similar phenomena occurs generally. In a hermophrodite, chromosome loss from the male cells of the developing organism produces a sector of haploid tissues. Each haploid cell later undergoes an equational meiotic division resulting in the formation of two sperms. During the development of the larva of the mosquito, *Culex pipiens*, there is an increase in chromosome number in the epithelial cells, lining the ileum, These cells are quite

large and function of their level of poloidy, and contain what have been called multiple complexes, or groups of chromosomes in close association. As metamorphosis proceeds, the number of the chromosomes in these cells decrease, and by the end of metamorphosis the chromosome number is appreciably lessened, and there are very few large cells. The mechanism of raduction is similar in some ways to meiotic division, *i. e.* the chromosomes pair and separate to the poles. In this way several divisions take place, gradually lowering the chromosome number. The haploid number of this species is 3, and the first division is characterized by the pairing of homologous chromosome with each group consisting of up to 64 strands. In anaphase the homologous strand separate from each other without any longitudinal division, so that daughter cells have reduced number of chromosomes. At the end of several divisions, the chromosome number is cut from 64n to 4n.

Somatic segregation—Normally the division of the cells results in two daughter cells that are genetically and cytologically identical. Occassionally there is a genetic or mechanical disturbance that lead to two unlike daughter cells, for example, nondisjunction causes the production of daughter cells with different chromosome number. Such a difference in somatic tissue is an instance of somatic segregation, which define any process in which somatic divisions give rise to cells differing genetically and or cytologically. Somatic gene mutation and somatic reduction may also produce tissue varients. The term mosaic introduced earlier, is generally used to the similar phenomenon in animals. Due to the somatic variation morphological differences are evident in different morphologies (colour, size, etc).

Endopolyploidy—Endopolyploidy results from endomitosis, or repeated duplication of the chromosomes with nuclear divisions In polyteny the chromosomes remain in association as multistranded, giant chromosomes. *The other type of endoploidy is polysomaty,* in which the chromosomes separate from each other within the nucleus. In polysomaty each chromosome is normal in appearance and however, the total polyploid number of chromosomes is distinuishable. The well documented cases of polysomaty is found in *Garris lateralis*. The diploid number of chromosomes is 21, one of which is the heteropycnotic X (sex) chromosome. It is easy to determine the number of sets of the chromosomes present in the cell only by counting the number of heteropycnotic bodies of the nucleus.

In *Garris*, chromosome number, as high as 1024n have been found in several somatic tissue.

Polysomaty also occurs in certain lepidopterans, specific cells of the grasshopper, and some plant tissues. The root cells of spinach often exhibit varying degrees of polysomaty, as do those of *Rhoeo discolor*. At leaset there are few organisms in which particular cells exibit polyteny and polysomaty simultaneously. Studies on the *Culex* suggest that polyteny arose first and that a longitudinal separation of the chromosomes followed, increasing the ploidy of the cell without affecting the polyteny (endomitosis of polytene chromosome). Polysomaty may result from some mechanism other than endomitosis. Most liver cells in female rats are diploid at birth, but the number of polyploid cells increases with age (especially during the first three months) Concurrently cells with two diploid nuclei and others with two tetraploid nuclei appear.Such binucleate cells arise through karyokinesis without cytokinesis, the nuclei fusing to form single polyploid nuclei in subsequent divisions. It has been noted that the changes in the number of chromosomes also occur during differentiation. Beerman embodied the possible bases for relationships between chromosome number and activity and differentiation. He states, "in synthetically active cells, growth is simply a means to increase productivity, which of course, would be impossible without a corresponding increase in the number of enzymatically sites in the nucleus, *i.e.* multiplication of the chromosome strand."

Induced Polyploidy—Polyploids have been induced experimentally by several methods in a number of different plants. Any mechanism that interferes in spindle formation and fission during mitosis might result in a doubling of the chromosomes. Maize and some other plants responded to the temperature treament, the chromosome number of certain cells was increased. Some of these cells gave rise to germinal tissue and whole plants were propagated. Other such cells were cultured artifically and polyploid plants were produced. Some other methods, were used in tomatoes such as decapitation. When the bud was removed, some shoots developing from the scar tissue were tetraploid. These were propagated and whole plants with 4n chromosomes were produced. The method of inducing polyploid has become most widely used. This was discovered by Blakeslee, Avery, and Nebel in 1937. They found that an alkaloid *Colchicum autumnale* could produce a disturbance in spindle formation during cell division. It

was noted that when growing root tips were placed in appropriate concentration of colchicum, the chromosome of treated cells duplicate themselves properly, but spindle formation was abnormal and cytokinasis did not occur. Restitution nuclei with all sorts of chromosome irregularties were produced in the treated tissue. Some cells occurred with the completely doubled chromosome number. When these cells were propagated, tetraploid plants were produced and tetraploid seeds were obtained.

POLYPLOIDY IN ANIMALS

The relative scarcity of polyploidy among animals presents a sharp contrast to its wide spread prevalence among plant, particularly in the angiosperm groups.This problem was easily considered by Muller (1925) who came to the conclusion that polyploidy would be quite limited among animals because the sharp separation of the two sexes rests upon a chromosomal number to some extent. The evidence bears out this hypothesis where polyploidy is known with certainty ,in the form of established races, it is usually intimately bound up with parthenogenetic mode of development.

Some polyploidy, however is present among certain animal groups, although the type of polyploidy in most instances remain to be established. White (1854) has published a number of histograms of chromosome numbers, based upon the tabulations of Harvey (1917, 1920) in which it seems quite clear that polyploidy of some sort has functioned to produce the variations in chromosome number that have been found. Thus in oligochaete worms which are hermophrodite, haploid number of 16 and 32 are most commonly found. In the *Hirudinea* the number 8 and 16 have been found together with others in between. The genus, *Mesostoma* of Rhabdocoela contain one species with a haploid number of two, six species with 4, one with 5 and one with 8. This would strongly suggest that the 4 and 8 chromosome species are tetraploid and octaploid but as White (1954) points out, such an assumption would require exact knowledge of the basic chromosome number for the genus and in many groups this determination becomes the virtual impossibility. White thus accepted the view of Slack (1938), Gates (1942) and Vandal (1938) that polyploidy has played a major role in animal evolution. Smith (1941, 1942) studied the sawflies which however has revealed an authentic case of polyploidy unassociated with parthenogenesis. Most of the species of *Neodiprion* and *Diprion* hove 7 chromosomes in the male and 14 in the females.

the male developing parthenogenetically as in other hymenoptera, *D. simile*, however, has 24 chromosomes in the female and 14 in the male. Meiosis in the male is similar to that found in hymenopteron makes, the first division is missing and the second is equational which in the female bivalents but not quadrivalents are formed. On the basis of above observations Smith considered, *D. simile* as allotetraploid, but it may be, as white suggests that the small size of the chromosome and their low chiasma frequency, precludes quadrivalent formation, thus observing what may well be a good case of autoteraploidy.

Although no known polyploid race has been established in the vertebrate group with the exception of the golden hemstar (White, 1954). Frankhanser (1938, 1939, 1945) pointed out that triploids and tetraploids are by no means uncommon as individuals in natural population of urodeles. Frankhanser and Hamprey (1950) have also bred a tetraploid female axolotle to a diploid male.

Polyploidy and evolution—It is a well known fact that polyploidy combined with inter specific hybridization provides a mechanism by which new species may arise suddenly in nature. Anderson has pointed out that this process is an important in the evolution of many plants, *Iris versicolor* with 108 chromosomes, was shown to have doubled chromosome complement of a hybrid derived from a 72-chromosome, *Iris* of the Mississppi valley and a 36 chromosome artic *Iris* from Alaska. Evidently these species, now separated geographically, have grown near each other at some time in their ancestral history. Müntzing working in diploid and tetraploid species of *Galeopsis*, found that one of the tetraploid, *G. tetrahit* (2n=32) could be synthesized from crosses involving the diploid species *pubescens* and *speciosa* (2n=16) *G. tetrahit* is not a strict amphidiploid but arose from a back cross between a triploid F_2 plant (2n=34) and one of the parent, *pubesense*. This increase in the number of the chromosome is due to the functioning of unreduced gametes. These observations and experiment varifications have substantiated and function of polyploidy in the evolutions of some plant groups. The most interesting aspect of such work are the duplications processes, that have been envolved in the origin of species.

Practical application of polyploidy—Although the induced polyploidy has not been exploited to a great extent yet its practical

applications may become more common. It has been found that by artificially induced polyploidy, disease resistance and other disirable qualities have been incorporated into some commercial varieties of plants. *Nicotiana tabacum* (the commerical tobacco) was susceptible to the tobacco mosaic virus, where as *N. glutinosa* appeared at first observation to be resistant. It has been further investigated, that in *N. glutinosa*, the virus killed the cell that they invaded and the particles become isolated in the dead cells. In this way the apparent resistance was attributed to hypersensitivity. When the two species of tobacco were crossed, the hybrid thus produced was found to be resistant to the virus but totally sterile. Many polyploids were selected and cultivated because of their large size, vigour, and ornamental values, before their chromosome number were known. Several varieties of grapes and cranbarries have also produced sports with giant fruits. Some of these show promise of practical usefulness.

SUMMARY

The normal chromosome constitution of higher plants and animals is the diploid condition with n pairs of chromosomes, each having the homologous partner or mate. The pairs are evident at meiosis. In many plants and animals, abnormality have been noted. The change occurs in the normal number of the chromosomes. These may be of the following types.

(A) **Change in the entire set of the chromosomes ; n=basic or monoploid number.** This may be of (a) **haploidy,** *i. e.* with n number of the chromosome or may be (b) **polyploidy,** *i. e.*, a representation of more than two homologous chromosomes such as **triploid** (3n), tetraploid (4n) ; pentaploid (5n) ; and so on. However, if the chromosome multiply from a single diploid and the homologous come from the same source as in pure strains or homozygotes it is termed as **autopolyploid** ; while **allopolyploid** forms are derived from a hybrid between the two diploid, so that the homologous come from two different sources.

(B) ***Changes involving the numbers of chromosomes in a set (heteroploidy).*** This may be of (a) **monosomic** (loss of chromosome from one set) when the process occurs in the diploid, the chromosome complement is 2n—1 or may be (b) **polysomic,** the addition of one or more chromosomes to one set. This may be **trisomic** =2n +1 : tetrasomic 2n+2, pentasomic 2n+ 3 and so on. The last type is (c) **Nullisome** which represent the loss of both chromosomes of a pair.

24

REPRODUCTION

One statement about living things that has no exception is: They can die. Death implies life, and life implies the possibility of death. This continuity of the life is brought about by the special process, known as **reproduction.** Reproduction, a primary characteristic of living, is the process in organism that result in the formation of new organisms of the same kind as the reproducing animal (organism), though usually with slight structural, physiological and genetical variations. Underlying all form of reproduction is the cell division.

The ability to produce new individuals is the basic fundamental characteristic of all plants and animals. Previously it was belived that the small animals like bacteria and protozoans arose from nonliving materials by spontaneous generations. For example worms and tadepole arise from mud and flies from the careasses of a deed animals. But this idea has been completely rejected with the development and recent advancement in biological sciences. Francesco **Redi**, an Italian scientist showed that the maggots and flies are produced from the meat when the living flies have laid their eggs on such materials. Later **Loius Pasteur** (1861) suggested that when the bacterial culture is heated to kill the organism, and properly stoppered to prevent reinvasion by germs or other spores from the air, they would remain without life. All these evidence, however, indicate that new life comes only from pre-existing life.

In single celled organisms, of course, cell division result in reproduction of the entire organism. In multicellular organisms, cell division is also essential for reproduction, but also serves other purposes as well. There are many methods of reproduction which may be classified into two main types, **(1) somatogenic or asexual** type and **(2)cytogenic or blastogenic type or sexual type.** In unicellular organisms and most primitive animals and plants, the asexual type of reproduction fairly occurs, while in multicellular organisms sexual type of reproduction occurs.

Somatogenic or Asexual reproduction

The somatogenic or Asexual reproduction is rare in nature. It may even be considered an an: :hronism, *i. e.* a form of reproduction hidden away in insingificant residue of primitive types. One might almost say that nature tested it as a means of reproduction, found it unsatisfactory, and so abandoned it.

This reproduction involves the sub-division of the parent body into two or more segments or parts. Each of the parts has *the power to reconstitute a whole new individual like the parent.* The sub-division in the body occurs either amitotically or mitotically. The somatogenic reproduction is carried out in different ways :—

(A) **By Transverse fission**—In most of the protozoans, the principal mode of asexual reproduction is transverse fission in which *body divides across the longitudinal axis of the cell.* The resulting daughter cells are unincleate which re-develop other necessary parts in due course of time. The larvae of many multicellular organisms like jelly fish and worms also divide by transverse fission.

(B) **By Longitudinal fission**—The longitudinal fission occurs in *Euglena.* This division is parallel to the longitudinal axis of the parent body. The nucleus divides amitotically into two daughter nuclei. In certain higher vertebrates and in a number of mammals for example armadillos and men, longitudinal fission takes place in the early embryonic stage resulting in the formation of true twins and true quadruplets from an original single parental embryo. In *Ceratium*, the plane of the division is oblique.

(C) **Multiple fisson**—In this type of division ; nucleus divides several times. It is followed by the cytoplasmic partition and consequent encirclement of each daughter cell. The nucleous divides either by repeated binary fissions, as in *Plasmodium* or by simultaneous multiple division, as in *Aggregata.* The parent body thus simultaneously divides into as many daughter individuals as there are nuclei. In a number of parasitic wasps and insects, the multicellular embryo sub-divides into many of cell-masses each of which capable to become complete individual. This process is quite common in Foraminifera, Radiolaria, Sporozoa, and also in certain mastigophorans.

(D) **By Budding**—The lower invertebrates also reproduce by the formation of the buds. The buds arise as an outgrowth from the body of the individuals. This outgrowth possesses a part of

parent nucleus. The buds so formed detach from the parent, grow to become adults. The budding may be simple if single bud is formed or may be multiple, if several buds are developed simultaneously. Budding can further be differentiated on the basis of development of bud gemmation. The bud may be Exogenous in origin if it develops outside the body as in *Ephelota*, *Mixidium* and *Noctiluca*, etc. or it may be endogenous in origin, if it develops inside the chambers called brood chambers, as in may suctorians.

(E) Regeneration—Another asexual mode of reproduction is by regeneration, which is common in plants but also occurs among many animals. The capacity to replace or regenerate parts lost by injury or otherwise is allied to growth after fragmentation.—Underground stems, leaves and branches readily regenerate the entire plants (roots are seldom able to do this). Fragments of many worms and of starfish, even as small as 1/200,000 of the volume of the original ornanism, can regenerate a complete individual. In higher animals where cells have become extremely specialized such regeneration does not occur.

(F) Gemmulation—This type of reproduction is generally found in sponges. Gemmule is the accumulation of the various kinds of cell. These cells mainly include archeocytes and thesocytes. The compact little belt-like structures are formed at the time of unfavourable condition when the parent body dies off. At the onset of favourable condition, each gemmule forms a new sponge. In some of the animals, similar asexual reproductive internal buds are formed which are generally known as statoblasts. They develop into new individuals.

(G) Plasmotomoy—This term was first of all introduced by Doflein for the division of multinucleate protozoans. In *Opalina*, *Pelomyxa* and *Actinosphaerium*, the cytoplasmic division occurs independently of nuclear division. The nuclei already present in parent are distributed at random into daughter individuals. These after sometimes regain the normal number of nuclei by further divisions.

Asexual reproduction which usually occurs through mitosis, undergo all the process of changes which take place on general mitosis described on page 190. In this process, the number of the chromosomes remain the same as present in the parental cell. There is no change in the chromosomal structure and their physiology.

Cytogenic or Blastogenic or Sexual Reproduction

The most common form of reproduction met with among the many celled plants and animals, as well as among large number of single celled organisms, is sexual reproduction—the formation of haploid sex cells (gametes) and the subsequent fusion of two such cells to form a zygote.

In primitive or early forms, fusion may occur ocasionally between two parents or more often between two gametes produced by the same parent. In the vast majority of organisms, however, such is not the case ; but instead, gametes are formed by different parents which tends to unite. This mode of zygote formation is termed **"fertilization"** and prevails in different plants and animals. Sexual mode of reproduction has evolved somewhat differently in plants and animals, and it is desirable to study different expressions of any one biological function because such expressions often reveal an evolutionary sequence. The sexual reproduction may be of two types, *i. e.* sporulation and gametogamy.

A. **Sporulation**—Ocasionally, single-celled organisms *e. g.* the ameboid parasites undergo a unique mode of reproduction in which the spores are formed. The spore is a miniature structure produced by mitosis from the parent cell. They are commonly mobile, some being provided with locomotary organs, such as flagella, with the help of which they travel in the water and air. This new cell or spore will in turn divides and redivides to form the multicellular body. The spores are generally formed to tide over the unfavourable conditions.

B. **Gametogamy**—In metozoa, sexual reproduction generally means the formation of special reproductive cells, *i. e.* gametes by the parents and the uniting of the two. These gametes (male and female) are formed by the process of meiosis. In meiosis (page 204) the new cells so formed (male or female gametes though gameto-) genesis have half the number of chromosomes to the parental cell. The crossing over however, brought about certain changes in the structure of the chromosomes which plays the major role in the biochemistry of the inheritence. In most primitive forms (plants and animals) mobile spores swim about actively and then pair, and finally fuse to form zygote. In higher and advanced forms there occurs specialization of sex cells. One gamete is the large passive female (egg) and other is small mobile gamete (sperm). The gametes possess the X-number of chromosomes, *i. e.* haploid in

condition and formed by the meiotic division from the diploid cells. The gametogamy can be further differentiated on the basis of shape, size and in character of gametes.

(1) **Autogamy**—In this type, the fusion gametes are derived from the same parent cell. This process can be differentiated from amphimixis. In the latter the nuclear fusion occurs between the nuclei of different individuals.

(2) **Isogamy**—When the gametes are similiar in size and form such gametes are called as isogametes and their union is isogamy. They may be produced by multiple fission and are generally provided with flagella. Although the isogametes are similar morphologically but they display a physiological differentiation of sex. Isogamy occurs in may protozoans.

(3) **Anisogamy or Heterogamy**—In the majority of cases the two fusing gametes differ in size, shape and behaviour. Such dissimilar gametes are termed **anisogametes** or **heterogametes** and their fusion is known as **anisogamy** or **heterogamy**. These anisogametes differ from each other in morphology and as well as in physiology. The smaller gametes are mobile and active, generally provided with flagella and long tail. They function as male gametes or microgametes. The larger gametes are passive, immobile, larger in size and termed female gametes are macrogametes. The microgametes correspond to the spermatozoa of metazoa and macrogametes to the ova. Anisogamy has been observed in Sporozoa, protocilliates and in many plants and animals.

(4) **Macrogamy**—Sometimes syngamy occurs between macrogametes of the species as in *Actinophrys*.

(5) **Microgamy**—If syngamy occurs between the microgametes, the process is called the microgamy. It has been observed in *Arcella*.

(6) **Other types**—There are also few other types of sexual reproduction such has **conjugation** and **hologamy**. Conjugation is the temporary union of two gametes or individuals of the same species during which they merely exchange their nuclear materials. Later on they are separated. This process only occurs in Protozoa (mainly in Suctoria and Euciliata). The two sexual individuals are termed as conjugation which may be equal (isogamous) as in *Paramecium* or unequal (anisogamous) as in *Vorticella*. In hologamy the gametes are like the parental cell morphologically but physiologically they function as gametes.

In many of the cases the artificial reproduction may be brought about by many of the external agencies—by which plants and animals are purified. In recent years it is the practice among scientists to produce the plants and animals by artificial propagation, so as to develop the more stable and better forms.

The sexes as we know them today in plants and animals, with their respective sex organs and gametes, did not in all likelihood make their appearance in nature all at once. Although their mechanism of reproduction were available, nature for various reasons apparantly favour. d the bisexual pattern now prevailing in higher animals and plants.

SUMMARY

Reproduction is a basic characteristic of all livings. Cells reproduce by either **mitosis** or meiosis. There are two main types of reproduction, *i.e.* asexual reproduction and sexual reproduction. The asexual reproduction may either be by fission (transverse fission, longitudinal fission or multiple fission), by budding, regeneration, gemmulation or plasmotomy. Asexual reproduction occurs through mitosis. In mitosis each new cell contains the same number of chromosomes as the parent cell.

The sexual reproduction is the characteristic of multicellular animals and higher plants. The sexual reproduction take place either through (a) sporulation (spore formation) or (b) gametogamy (gametes formation). The gametes are or two type (1) male gametes and (2) female gametes. The gametes formation process is known as gametogenesis. In gametogenesis reduction division occurs through meiosis. In meiosis haploid gametes are formed with the change in the structure and physiology of the chromosomes due to the crossing over and linkage.

25

FERTILIZATION AND CLEAVAGE

The term *fertilization* denotes the sexual union of two gametes, regardless of their relative structure, behaviour and the nature. One gamete (female) is large and apparently passive while the other (male) is small and active.The fusion product of the two is known as zygote. Strictly speaking the word fertilization denotes the process of making the egg fruitful, *i. e.* to develop the egg by sperm contact and as such may not always imply a fusion of sperm with the egg. The use of the word 'fertilization' in the sense of initiating the development reaches back to the dawn of recorded history. However, Leeuwen Hooke for the first time put forward a thesis that the egg must be impregnated by the seminal animalcule (sperm) in order to become fruitful.

The phenomenon of fertilization involves so many physiological and morphological processes starting from the maturation of gametes leading to the fusion of the male and female pronuclei. It leads to the cell division (cleavage) and formation of new individual. It also leads to the combination of maternal and paternal groups of chromosomes in the nucleus of zygote. Conviniently fertilization can be studied under the following heads.

1. The movement of sperms towards the ovum.
2. The penetration of sperm into ovum.
3. *Fusion of gametonuclei.*

1. **Movement of sperm**—Fertilization can be internal or external.In the former the sperms are dropped inside the body of the female and then they move onward to fertilize the egg. In the later case the sperms are dropped near or over the eggs which come of the female's body at the time. Normally for internal fertilization, copulation takes place which ensures the proper dropping of the sperms inside the body. The sperms move toward the unfertilized eggs. In the movement tail of the sperm helps much. It was believed that there exists some chemical attraction or force between the two (sperm and egg) which causes them to come nearer. But now it is

presumed to be purely accidental. Some of the eggs are protected by a jelly-like envelop and the sperm is required to penetrate this layer before it can touch the vitelline membrane. In the eggs of starfish (*Asterias*), this envelop is so thick, that further independent progress of the sperm is actually blocked because of this. In this case, beneath the point of contact, the ovum forms a small fertilization cone from which fine protoplasmic filaments grow out to trap the sperm. These filaments are then withdrawn to the fertilization cone along with the trapped sperm. In the process sperm has been observed putting some resistance. In other types (*Arbacia*) only the cone is formed which engulfs the sperm head, while in still others no fertilization cone has been observed. During the movement of the sperm towards the ovum, some lytic substances are reported to be secreted which enable the sperm to move and to blow through the getatinous envelop and cellular barriers to the surface of the egg. The movement is undoubtedly aided by the movement of the tail in most species.

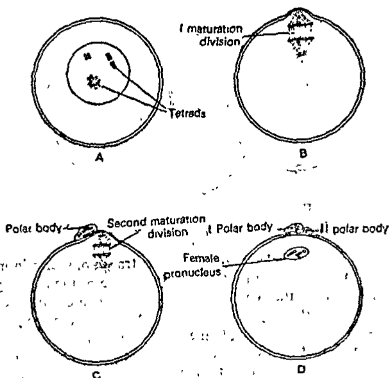


Fig. 167. Maturation of ovum

2. Penetration of the sperm—Previously it was believed that this process involves the fusion of the plasma membranes of the gametes. In some of the cases oogenesis is completed only when the spermatoza enter the oviduct such as mouse, and certain insects; polar bodies also developed after the enterance of spermatoza. Sometimes the ova develop directly only by coming in contact with spermatozoa. The portion of the sperm that first touches the egg is determined by the position of acrosome which is usually made up of lipoprotein.

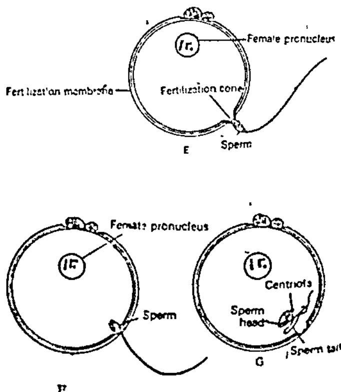


Fig. 167. Diagrams showing the penetration of sperm into the ovum.

How the egg behaves during the entrance of sperm in marine annelids has been studied in detail. All, but one of the many sperms which may have attached themselves to the egg are usually carried out from its surface by a jelly which flows out from an alveolar zone just beneath the membrane. From the inner region of the egg a transparent fertilization cone then extends across this zone and touches the membrane at the point where the sperm is about to penetrate it. Perforatorium pierces the membrane and the

sperm becomes attached to the one cone which is then withdrawn towards the inner region of the egg. In *Nereis* only head panetrates, middle piece and the tail are left outside. In sea urchin both head and middle piece enter while in most animals the whole sperm passes in.

In the different animals, there is considerable variation in the stage of egg maturation, at the time when the sperm enters. In sea urchin and starfish both divisions have been completed, thus sperm enters a fully matured egg. In frog's egg, the sperm enters during the metaphase of the second meiotic division. In some annelids, insects and molluscs, the penetration of the sperm occurs during the first metaphase. It may enter still earlier as in *Ascaris*.

Lillie (1913,14, 15) and Jush (1919) have provided the evidences to suggest that there exists a colloidal substance '*fertilizin*' in the egg which activates the sperm to enter the egg ; and the action of fertilizin is either counteracted or neutralized by the sperm after it has entered the ovum.

It is further believed that acrosome of the sperm has a perforating function which enables the sperm to pass through the egg membrane. But in the light of recent researches it appears untenable. Bowen (1924) admitted that acrosome has some mechanical role but he emphasized that it is essentially a secondary product ; its main function is to initiate the physico chemical reaction of fertilization. Usually only one sperm enters an egg. In some forms, likely vertebrates, insects, etc. polyspermy has been observed. However, in such cases only one sperm fuses with the egg nucleus and the others which have entered assist in the absorption of yolk and later on they degenerate. Once fertilized, the egg can not be returned to the unfertilized condition nor it can be refertilized again.

Fusion of gametonuclei—The enterence of the sperm into the

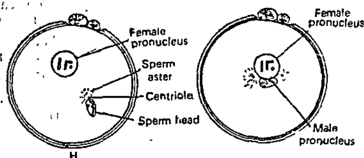


Fig. 168. Karyogamy of the ovum.

egg substance, its migratory movement into the ooplasm, its meeting with the egg pronucleus and then the fusion of the pronuclei afford an interesting problem. The factors governing the movement of the female and male pronuclei are unknown.

When the sperm nucleus has entered the egg, it begins to enlarge and becomes male pronucleus. Meanwhile the egg nucleus has matured to become female pronucleus.The male pronucleus often assumes the size similar to that of egg nucleus. The two now move towards each other and finally fuse to form the *zygote or fusion* nucleus with diploid nucleus.In *Ascaris* there is no true fusion of the two pronuclei ; but on meeting the nuclear membrane of the two disappear and the chromosomes of the two immediately enter the first cleavage, the next stage after fertilization.

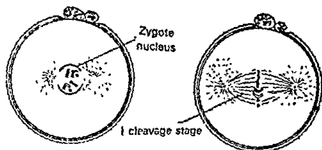


Fig. 169 Start of the first cleavage in *Ascaris*.

In the case of normal fertilization, it is clear that two main conditions are to be fulfilled. The first is the activation, by which certain physiological processes are set in motion or greatly accelerated. In most cases this leads into an immediate development of the cell into a new individual but in some animals and plants, the cell develops certain protective coats and enters into a dominant stage from which it emerges later under the appropriate environmental conditions. In either event there is a great physiological changes at the time of syngamy or fertilization. The complete activation may be induced by various physiological, physical and chemical treatment even in the absence of sperm. Furthermore some eggs show parthenogenesis (undergoing complete development without syngamy). The egg has accordingly been termed as independently activable system which contains everything necessary for development.

The second important effect of fertilization is diplosis, *i. e.*

the doubling of the number of chromosomes by the union of the two gametic nuclei. In fertilization or syngamy two genomes with the monoploid chromosomes number are combined into a diploid chromosome component. Every chromosome of this complement divides equationally at every somatic mitosis in the development of the resulting new individuals, so that every nucleus in this contain a descendent of every chromosome originally present in the zygote.

Polarity of the ovum and cleavage—After fertilization, the egg differentiates into two poles. The side towards the sperm penetration is the anterior side and opposite is the posterior side. The

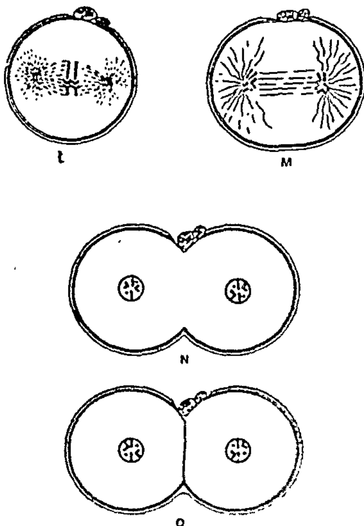


Fig 170 Scheme of first cleavage.

dorsal animal pole is physiologically more active than the ventral vegetative pole. This activeness is due to the absence of yolk in this region and a greater streaming power of protoplasm into it. Furthermore the egg of the different animals differ greatly in the amount of location of their yolk material. The two features, exert a strong influence upon the determination of various cleavage patterns found in the different classes of the animal.

Geometrically, the most regular cleavage pattern is found in egg having their yolk uniformly distributed throughout the egg as found in echinoderms. Such eggs are called the homolecithal eggs. In such eggs the cleavage is holoblastic. The first cleavage in such a type of egg is meridional (through the two poles), the second meridional but at right angle to the first,the third equatorial and the several following division in such planes take place and as a result of this, spherical mass of cells (blastomere) is formed. As development proceeds this sphere becomes a hollow blastula and this is in turn converted into gastrula.

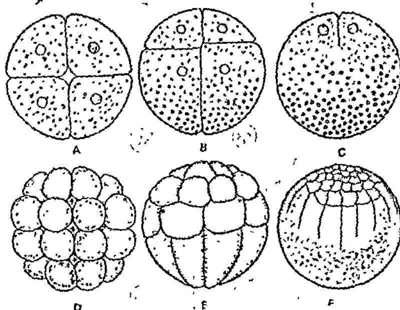


Fig. 171. Three types of cleavage in animal eggs,
A—C. sections of eggs in early cleavage stage.
D—F. surface views of later stages.

The egg of the frog is moderately 'telolecithal', *i. e.* its yolk tends to be denser in the region of the vegetal pole. The first and

the second cleavage division occur as in the homolectithal egg, but the third division is unequal, thus giving rise to eight unequal cells ; four of which are smaller cells (micromeres) at the animal pole and four larger cells (macromeres) at the vegetal pole. From this stage the macromeres divide at the lower rate and micromeres with the faster rate. Because of this first **blastula** is formed which is then converted into **gastrula**. The egg of Amphioxus and of the eutherian mammals are exceptional in having minute amounts of yolk. For this reason, though the eggs show polarity, they are termed **micro-lecithal**. As the quantity of yolk is minute, the egg undergoes complete cleavage, *i. e.* **holoblastic**. The first two divisions are meridional the second being right angle to first. Thus more or less four blastomeres are formed. The third cleavage is latitudinal and the plane lies slightly above the equatorial region. This gives micro and macromeres.

The eggs of bird, reptiles, squids and bony fishes and some lowly mammals are very strongly telolecithal. This type of egg is known as **meiolecithal**. This type of eggs are very densely packed up by yolk, but that is absent from the small germinal disc at the animal pole. The cleavage is, however, restricted only to this relatively thin layer of protoplasm and does not extend throughout the bulk of the cell. After a few divisions have occured, the young embryo has the form of the plate of cells, the **blastoderm**, lying against a large yolky mass. This is called the **mesoblastic cleavage.**

Still another type of cleavage has been noted among the insects. The first few cleavages in the fertilized egg are not accompanied by cytokinesis, the embryo being coenocyte during its earlier stages of divisions. As soon as the primordial germ cell has been set apart; the multiplying nuclei in the somatic region of the embryo, move to the periphery along with small masses of cytoplasm. The central region holds most of the yolk (**centrolecithal**). Cytokinesis occurs between the peripheral nuclei but the cells so formed remained open outside towards the yolk. Further divisions of these cells produce the ventral plate from which the most parts of the embryo arise.

This is, therefore, quite clear that there is some definite correlation between the type of cleavage and certain visible feature of yolk. The correlation is so far from complete, that this feature can not be regarded as more than a contributing cause of cleavage patterns.

The most important aspect of cleavage is the relation which

it bears to the internal differentiation of the embryo. It is further noted that this differentiation has proceeded much further in some animal at the given stage of cleavage. In *Clytia* 16 blastomeres have produced a complete embryo whereas in *Beroe* (Ctenophora) it has been observed that larva is incomplete, if a portion of the egg's protoplasm has been removed even before the first cleavage. Thus in latter case the eggs have a definite **promorphology,** *i. e.* it has developed an internal organisation which in some manner foreshadows the morphology of the young embryo. In other words the embryonic differentiations starts in the uncleavage egg. So with this point of view, the three processes which are closely associated and overlap each other (meiosis, syngamy and cleavage) we now add a fourth, *i. e.* embryonic differentiation.

It is now possible with the help of some deductable substances to study the internal differentiation in some eggs. The further discussion on the problem will lead us into a fascinating but extremely difficult field of developmental mechanisms.

Origin of Polarity in the egg—From the above description, it becomes evident that the eggs of vertebrates have acquired a distint polarity even whilst they are in the ovary. It is evident from the fact, that the yolk is always deposited towards one pole, *i. e.* vegital pole, and the nucleus lies nearer to the other pole, *i. e.* animal pole. Further in vertebrates, the end by which the egg is attached to ovary becomes the animal pole where as in invertebrates the end which faces the coelomic fluid, *i. e.* non attached end becomes the animal pole. From all this, it appears that the animal pole develops in the region of higher oxygen tension and the polarity of egg therefore is not due to the factors inherent in the egg but is induced by factors outside it.

SUMMARY

The term fertilization denotes the sexual union of two gametes, regardless of their relative structure, behaviour and the nature. The fusion product of the two (male and female gametes) is known as zygote. In real sense the word fertilization denotes the process of making the egg **fruitful,** *i. e.* to develop the egg into individual. The phenomenon of fertilization involves so many physiological and morphological processes starting from the maturation of gametes leading to the fusion of the male

and female pronuclei. The fertilization may occur in the following heads. (a) The movement of the sperm, (b) the penetration of sperm into ovum and (c) fusion of gametonuclei. The great details have been studied by different scientists, how the egg behaves during the enterence of sperm in it. Lillie (1913, 1914 and 1915) and Jush (1919) have provided the evidences to suggest that there exists a colloidal substance "fertilizin" in the egg which activates the sperm to enter the egg.

After fertilization, the egg differenciates into two poles. The side towards the sperm penetration is the anterior side and opposite to it is the posterior side. The dorsal animal pole is physiologically more active than the ventral vegetative pole. The cleavage may be of **holoblastic** (cleavage in homolecithal eggs) or **meroblastic** (cleavage in the *meiolecithal egg*). In the *telolecithal* egg the cleăvage is holoblastic. In certain cases of insects, abnormalities in the cleavage occurs. In certain cases, the first few cleavage in the fertilized egg are not accompanied by cytokinesis, the embryo being coenotype during its earlier stages of division. Later on, as soon as the primordial cells have been set apart, the multiplying nuclei of the somatic region of the embryo, move to the periphery along the small masses of cytoplasm. The central region holds most of the yolk (centrolecithal). Cytokinesis occurs between the peripheral nuclei but the cells so formed remained open outside towards the yolk. The most important aspect of cleavage is the relation which it bears to the internal differentiation of the embryo.

26

CELL DIFFERENTIATION

The differentiation of cell is one of the most interesting and universal phenomenon of the living world that occurs during the development of an individual. Cell differentiation means "a cell becoming different. A cell, is one of the fundamental structural and functional unit of the living matter ; the units by which the body of adult organism is built, are by no means the same. There are at least hundred or so distinctly different kinds of cells in man, yet in the first few days of the development, from the fertilized egg, there are only a few (perhaps two or three) different kinds of cells. From where these 90-odd additional cell kinds have come. This is the problem of great interest today. Perhaps no cell ever "*marks* time" in the strict sense. Sooner or later it changes. It becomes different with the passing of time, and two or more cells become different from one another. There are many ways in which cells become different.

Every cell possesses special equipment in the form of its genome—collectively the genes on its chromosomes in its *nucleus*—which the cell has inherited from its parent cell. The genome may be thought as a set of the instruction used not only in guiding the day-to-day activities of the cell but also in implementing the progressive changes in these activities—changes are also stages or steps in the process of cell differentiation. It should be kept in mind here that not only the genome is the source of instructions which a cell receives from its parent but the cytoplasmic continuity of the cell propagation also provides the basis for a hereditary mechanism, which parallels that located in the nucleus. In some kinds of cells it has been demonstrated that cytoplasmically localized instructions do play a role in cell behaviour.

However, one can distinguish three major categories of change: differentiation in time, differentiation in space, and differentiation in shape.

Differentiation in Time—This differentiation occurs with the change of time. If some one watches any particular *region* of an

egg it will be found gradually to change in character as time goes on. First it is a part of the general substance of the egg. But as the egg undergoes cleavage into many cells the region will become provided with its own nuclei and cell membrane and however, become the mass of cells, but at first these cells are of a very general kind. A little time later the cells will begin to form some particular tissue of the adult for example part of the muscle, of a bone, or a kidney, and so on. In doing so, the cells must change in chemical composition, a muscle cell must develop the contractile proteins of the muscle fibres, and bone cell must lay down the bone substances, and so on. Therefore, there is a sequence of changes as time passes, by which this region of the egg gradually acquires the characteristic of some specialized tissue of the adult. Since this process result in the production of an adult tissue, it is technically referred to as **histogenesis.**

Differentiation in Space—At the time of fertilization, the egg is more or less similar (though never exactly the same) in all its parts. It is still usually pretty uniform when it is divided into a number of cells and becomes a blastula. At the later stage of its development, however, one part of it will become the brain, another part an intestine and each of them will again divided into their respective components. There is an obvious production of many more different regions of the embryo than there were to start with. As a matter of fact, eggs at the time of fertilization are not entirely uniform throughout their whole mass. They always start with some regional differences between one part and another, but at first there are very few differences and very slight and however, difficult to detect them. In the later stages there are many more regional differences. Not only this but they are more striking and well marked. The process by which the egg become divided into many distinct different region is known as **regionalization.**

Differentiation in Shape—Eggs have a very simple shape, often spherical. The organisms into which they develop have, of course, complicated shape. Not only is their overall external surface molded in the structure of trunk, leg, head, tail and what you have, but internally they contain many different organs, each with a rather constant and particular shape. The process in which these changes in shape occur are known as **morphogenesis.**

If we take the cell in consideration, the differentiation of cell may be of two types, *i. e.* intracellular and intercellular.

(1) **Intracellular differentiation**—It includes the progressive internal changes which the cell undergoes in becoming different with the passing of time. Intracellular differentiation are found in various unicellular organisms, particularly during reproduction and regeneration. However, the diversification and synthesis taking place within unicellular organisms have thrown light not only on differentiation in these forms but also on intracellular differentiation in the cells of multicellular metazoans. All cells undergoing development show intracellular differentiation. The formation of an egg in the ovary and the sperm in the testis is the most extreme examples of intracellular differentiation. There is a wide spectrum from simple to complex cell specialization.

2. **Intercellular differentiation**—Intercellular differentiation is quite a different aspect of differentiation. In this process there is a progressive appearance of different types of cells in a population of cells. In other words, it is the process by which two or more cells *become different from one another.* The *clear example of this type* of differentiation is found in the early development of multicellular organisms.

Mechanism of cell differentiation—Why and how cell differentiation occurs : this question has not been adequately answered till today. We can examine some facts and point of views which probably will have to be taken into account if a satisfactory and comprehensive answer is to be found out. However, the knowledge of mechanism of intercellular differentiation is lacking in precision,

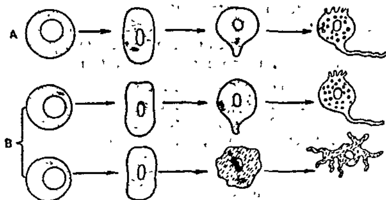


Fig. 172. Diagrams showing the intracellular differentiation (A), and intercellular differentiation (B).

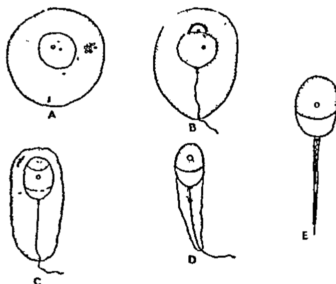


Fig. 173. Diagrams showing the extreme example of intracellalur differentiation in human sperm.

yet many observations and experimental results reported by experimental embryologists have hinted at the nature of some of the mechanism which operate at the cell population level. Interesting suggestions as to the mechanism of intracellular differentiation are linked to recent advances in our understanding of gene action. Since intra and intercellular mechanism are so closely linked, so interdependent ; it seems that a knowledge of both the type of mechanism will be necessary before the problem of "how and why cells differentiate" can be solved.

General way of differentiating cell—There are two general ways in which diverse types of cells may be formed during embryonic development. (1) By inheritance of different kind of cytoplasm in the egg : (2) By response to different microenvironment. In inheritance properties which distinguish the cell from one another have been cytoplasmically inherited, whereas in the second case, the distinguishing properties of the cells have been environmentally acquired. Theoretically, it would seem that inheritance of different kinds of genes would constitute a third general way of differentiating cell, but proofs are lacking.

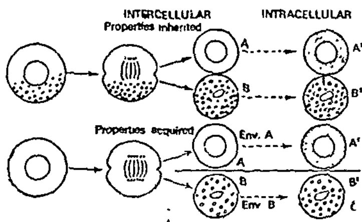


Fig. 174. Two methods of inter and intracellular differentiation.

The two general methods of cell differentiation have been illustrated in the above fig. 174. In the upper part of the figure the two A and B cell types have been shown which differ from each other, because of their cytoplasmic inheritance. The undergoing further specification or intracellular differentiation is in response to the diverse instructions which they have inherited. In the lower part of the same figure the A and B cell types differ because of their diverse environment consequently they differentiate further in the development in response to their diverse environments. However, it is probable that *intracellular differentiation in most cells* is guided by the interaction of inherited and environmental instructions.

Cytoplasmic inequality of the early blastomeres—In harmony with the differences in the location and activities of the various blastomeres of the cleaving egg it is apparent that the differences exists in the cytoplasmic or ooplasmic substances within the various cells in many species. In the case of frog, the quantity of the yolk substances present in the cells to the yolk pole is much greater than that of the animal pole. In the same way, in foured celled stage the substance of the grey crescent is located in two of the blastomeres, while the other two have little or none of this substance. The two cells, out of the four, are qualitatively different from the other two. In the case of Ascidian, *Slycla partita* the presence of the yellow crescent, *yolk substance*, and *grey crescent* materials demonstrate that in the four or eight celled stages,there are qualitatively differences

n the ooplasmic substances which enter into the composition of the respective blastomeres (Conklin 1905). Similar conditions may be demonstrated for *Amphioxus*, although pigmented material is not present in the egg Conklin, (1932). As the development proceeds in the egg, a progressive difference in the cytoplasmic substances become evident.

Not only this, but the differences in the developmental history of the cell or cells is determined by the presence or absence of a specific ooplasmic substance in the blastomeres, as has been shown experimentally in many animals. Spemann (1902 and 1903) and (Ruud, 1925) demonstrated in the amphibian embryo that each of the blastomeres of the two celled stage will develop a complete embryo if the first cleavage plane bisect the grey crescent. If however, the first cleavage plane is right angle of the median plane of the embryo, the blastomeres which contain the substance of the grey crescent will develop a complete embryo, where as the other one will give origin to a very imperfect form which does not gastrulate normally to produce a semblance of a normal embryo.

Certain, similar experiments upon the egg of the newt, *Triton palmatus*, indicate that a marked difference in the "developmental potencies exists between the dorsal and ventral sides of the egg within a few minutes from fertilization. Here, however, the formation of the grey crescent seems to be the secondary phenomenon which makes this difference clearly visible in the egg of some species".

In the case of *Amphioxus*, similar evidences are obtained after the blastomeres have been mechanically isolated. Typical embryos are developed always from the first two blastomeres. These twin embryos are half of normal size. In the same way the right and left halves of the four-celled stage also give rise to normal larvae. Moreover, blastulae also develop from isolated blastomeres of the eight cell stage, but the blastulae which develop from the micromeres are smaller and have only one type of cell namely ectoderm, and however, they never go further than the blastular stage. On the other hand, those from the macromeres are larger and have ectoderm, mesoderm and endoderm but they never progress further than the gastrular stage of development. It is due to the fact, that the macromeres contain potential mesodermal, endodermal and ectodermal ooplasm, where as micromeres lack the mesodermal and endodermal substances and contain only ectodermal material.

In the case of *styela* egg; a somewhat different condition is found. If two cleaving egg of this species is separated at the two cell stage into two separate blastomeres, each blastomeres develops only one half of an embryo. That is, the right blastomere develops

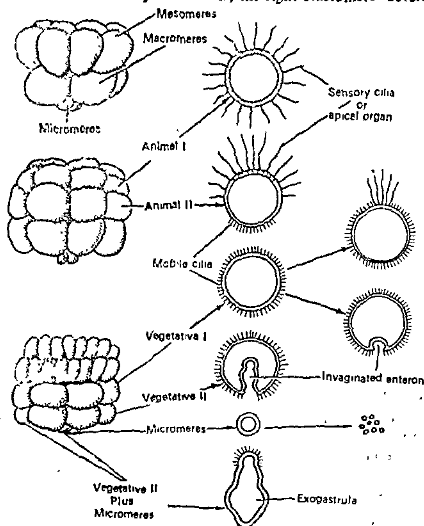


Fig 175. Diagram showing the developmental potencies (cell lineage) of isolated blastomers.

an embryo minus the left half, while the left blastmere produces the opposite condition. There is some tendency to develop or regulate into complete embryo in that the ectoderm grows over the half of the embryo which failed to develop. Similarly at the four-cell stage, isolation of anterior and posterior blastomeres gives origin to anterior and posterior half embryo respectively

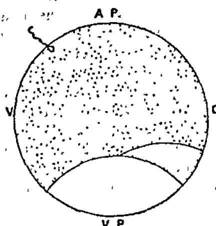


Fig. 176. A fertilized amphibian egg with localized grey crescent cytoplasm opposite to the point of sperm entry.

The pattern of quantitative segregation of the mitochondria

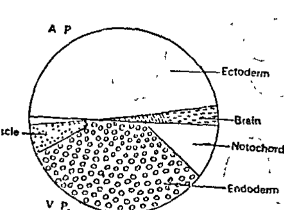


Fig. 177. Diagram of a fertilized egg, showing sharply localised cytoplasmic regions.

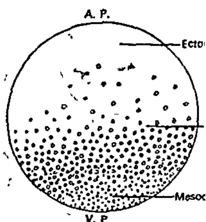


Fig. 178. Hypothetical gradient type of cyto-differentiation in sea urchin egg.

has significant effect on cell differentiations. The cell or cells, receiving the largest number of mitochondria seem to have greater capacities for further differentiation in comparison to those which receive fewer mitochondria. This has been demonstrated by isolating the various types of cells. Although not unequivocally established, it seems an interesting probability that the segregation of mitochondria, which are "energy-furnishing plant" of the cell

and thus integral parts of its synthetic mechanism might be a significant mechanism for diversifying the blastomere population. It is well known fact that many kinds of eggs possess specific cytoplasmic regions which play profound role in the subsequent behaviour of cell cleaved out of these regions. The role of cytoplasmic regions in subsequent cell behaviour has been demonstrated by the abnormal pattern of differentiation in egg, such as those of the ascidians or tunicates following displacement of the tissue specific or organ specific cytoplasmic region by centrifugation. However, strong centrifugation prior to cleavage displaces entire cytoplasmic region and result in bizarre with their different cell types and organs out of all proper relation to on e another. There are however,

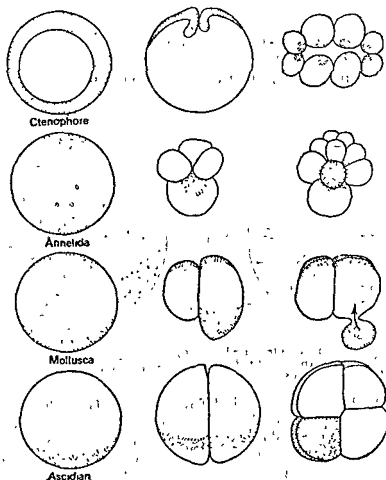


Fig. 179. Patterns of blastomere differentiation.

evidences that the important cytoarchitecture of many eggs is located in the cortex, the region just below the surface of egg and that centrifugal displacement of the relatively tough or stiff cortex, in contrast to the more fluid interior, or endoplasm, is relatively difficult. It has also been demonstrated that removal or injury of parts of the cortex of certain eggs (for example, amphibian, and squid) results in defects in the later developmental pattern. Although very little is known about the cortical architecture of an egg, however the election microscopy has not yet revealed its significant pattern—it is probable that further study will reveal it to be as highly and complexly structured as that of the ciliate protozoans It is a well known fact that in ciliates the cortex plays a profound role in the intracellular differentiation. It is perhaps significant that elements of cortical pattern are present in all plant and animal groups.

Although the intercellular differentiation resulting from the inheritance of cytoplasmically different regions of the egg probably occur to a varying degree in all animal species during the cleavage phase of development. But however, it is not certain to what extent such a method of intercellular differentiation continues in latter stages. The supposition, based upon other observations indicates that it is supplanted in large parts by the second type of mechanism. This does not mean that those distinguishing properties which cells have inherited by all cells as in bias—a tendency which can be reinforced, modified, blocked or even reversed by influences external to the cell. In some cells the bias may be so great that no external influences, can change it. In others, the final decision as to the cours: of further differentiation may be made outside the cell. In the latter cell inheritance would still set the limit to their differentiative capacities and perhaps favour one of these. It seems unlikely that cytoplasmic inheritance would be totally natural, for this would imply that the egg from which the cells were derived as cytoplasmically homogeneous.

It is quite clear from the account that during the early cleavage stage many different animal species, a sorting-out process is at work which segregates into different blastomeres having distinct ooplasmic substances which possesses different developmental potencies Thus segregation of different substances into different blastomeric channels is one of the function of cleavage.

NUCLEAR EQUALITY—

It has long been known that the nucleus and its genes play an indispensable role in the daily life of a cell, but the question is whether the nucleus is of any significance in the process of cell differentiation ? Genes do play a decisive role in implanting the intracellular differentiation but the general controversy is that what, if any, role do the gene play in intercellular differentiation, *i. e.* in causing or initiating diversification in the cell population ?

Although acceptance of the principle of genic equivalence of daughter cells resulting from the mitotic division of a parent cell is reasonable in view of all we know about cell division. But to assume that genic or nuclear equivalence persist throughout the life cycle of a multicellular organism is not so reasonable. The following description will make it obvious that what role, the nucleus play in differentiation.

1. **Developmental equivalence of cleavage**—We can not speak with finality about genic equivalence of embryonic nuclei but must resort to use of expression. Developmental equivalence of the nuclei by meant that two or more nuclei whose role in a developmental appears to be identical.

A precise and illuminating experiment demonstrating nuclear equality of the early blastomeres may be performed by the hair-loop contriction method (Spermann, 1928 ; Fankhauser, 1948.) For example the fertilized egg of the newt, *Triturus viridescens*, may be constricted partially by a hair loop so that the zygotic nucleus is confined to one side (Fig. 179 A and B). The side possessing the nucleus divides but the other side does not divide (Fig. 179 B and C). Then by releasing the ligature between the two sides at various stages of development of the cleaving side, *i.e.* 2-, 4-, 8-,16- and 32-cell stages, a nucleus is permitted to "escape" into the cytoplasm of the uncleaved side (Fig. 179. C, E ; in D the escaped nucleus is seen in the blastomere to the left). Further, by tighting the loop after the escaping nucleus has entered the uncleaved cytoplasm, further nuclear "invasion" of the nucleaved part is blocked. If, however, the original constriction was made so that the plane of canstriction coincides with the plane bilateral symetry *i. e.* if it constrict the grey crescent into two halves, the result is two normal embryos. This occurs after the 2-, 4-, 8- and 16-cell stages of the cleaving half of the egg. Nuclei permitted to escape when the cleaving has reached to 32 cell stage do not produce normal

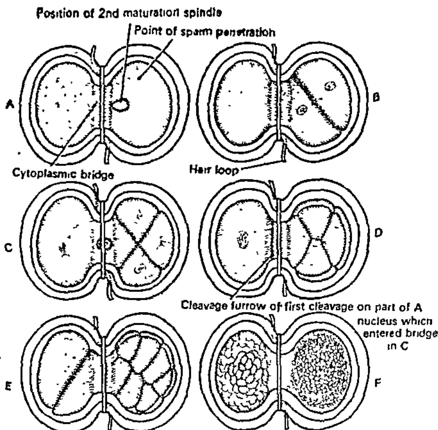


Fig. 179. Showing of cleavage of a partially constricted egg illustrating delayed nucleation.

embryos in the uncleaved side, probably because of the changes which have occured in the meantime in the cytoplasm of the uncleaved side and not to the qualitative differences in the nuclei at this stage.

Another type of experiment upon the early cleaving blastomeres which demonstrates nuclear equality has been shown by Wilson (1925) that a cleaving egg when pressed between two glass surfaces will divide parallel to the pressure surfaces. This is, the mitotic spindle is moved into a position parallel to the pressure surface. Under these condition, the spindle obeys the second law of Hertwig, namely, that the mitotic spindle tends to coincide with the long axis of the protoplasmic mass. Cleavage under pressure so applied, therefore, will result in a series of vertical cleavage planes. In the sea urchin Fig. 180 if pressure is applied in the four cell stage the mitotic spindle will form in a horizental position(Fig 180 E) in-

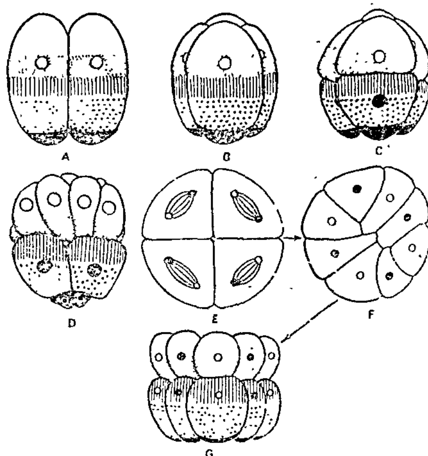


Fig. 180. Diagram showing the nuclear equality in sea urchin egg.

stead of in the vertical position (Fig 180 B and C) where no pressure is applied. In other words, all of the nuclei in the upper blastomeres (Fig. 180 C) will be displaced horizontally by the applied pressure (Fig. 180 F). If however, the pressure is released at this stage, the mitotic spindle again obeys Hertwig's rule and forms in the long axis of the cytoplasm which is now vertical in position. As a result, upper and lower cells are formed (Fig 180 G). It is quite clear from this figure that there is a mixture of these nuclei after the pressure is released. Regardless of this redistribution of nuclei, development proceeds almost normally. Development thus appears to be governed by the presence of special ooplasmic substances contained within the respective blastomeres (Fig. 180 A to D).

The evidence from the foregoing experiments suggests the conclusion that the nuclei in the early blastomeres are qualitatively equal.

2. **Equivalence of post-cleavage Nuclei**—Small fractions of chick and duck, egg, in the early blastoderms (approximate 5000 to 30,000 cells) can develop into small but complete embryo bodies. *None of the cell in some of these fractions would have normally* participated in forming embryo body tissues. They normally would have become extra embryonic (amnion and chorion) type cells. It is this significant that thousand of nuclei in cells of the blastoderm are developmentally equivalent.

Quantitative and qualitative cleavage and their influence upon later development—In one experiment Driesch (1892) shocked an early blastomere of the sea-urchin. He found that the two blastomeres resulting from the two division continued to divide perfectly and rightly. The first division of the isolated blastomere was meridional as if it had retained contact with its mate of the two cell stage. The next division was latitudinal also, as if it had retain contact with its original male. Further he noted that isolation of cell after two cell stages causes the later development imperfect and abnormal. The isolation of blastomere is the eight cell stage of development, in most cases, result the abnormal development. The experiments with the *Amphioxus* (Conklin 1933) give the same results. Thus it can be suggested that the division of early egg is purely a quantitative.

Initiation of Differentiation—It is, however, difficult to understand the complete mechanism of intracellular differentiation without an accompanying reference to the initiating causes of this response. It certainly seems that the initiating cause of the differentiation of many *kinds of the cells lie outside the cells. Even in cells* which have cytoplasmically inherited their distinguishing properties, *i. e.* blastomeres of some eggs, the pattern of cyto differentiation in the egg must have been formed in response to the microenvironment of the developing egg in the ovary. No completely endogenous (internal) mechanism, capable of making final decision throughout the life cycle of the species seems to exist anywhere. Thus so important is the microenvironment of many cells in guiding their differentiation.

Differentiation of cellular microenvironment—In the case of a cell population increasing in size equally in all directions by the proliferation of the cells, the individual cell would automatically occupy

different positions ; some would be on the inside, others at the surface of the spherical aggregate.

The local environment of the inner cells would be different from that of the outer cells because among other differences resulting from the production of cellular products, the inner cells would

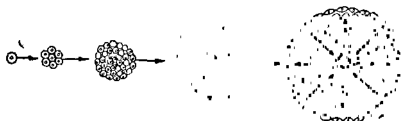


Fig. 181. Hypothetical aggregate of cells showing divisions (Arrows show—inside and out side microenvironment differential).

not be directly exposed to the environment external to the aggregate as are the outer surfaces of the outer cells. Thus the cells in various intermediate positions would occupy still different microenvironments. An important feature of the development of the microenvironmental pattern in such a cell population is the fact that it would initially be gradient in form. Thus, certain components surrounding the cells would reach their highest concentration at the centre of the aggregate and gradually diminish toward the surface. Others would be distributed in gradient fashion in the opposite direction, *i. e.*, the concentration would decrease from the surface toward the centre. In this way the gradient distributions of substances both inside cells and inside cellular aggregates are one of the simplest manifestations of pattern and found in the early development of many species. When however the microenvironmental pattern of the aggregate becomes sufficiently diverse, the positions of the cells in the pattern would become important as they will respond by undergoing changes of one sort or another. These might be changing in shape, rate of mitosis, synthetic activities, etc. In this way, all over environmental pattern of the aggregate and different microenvironments, would become different from what they were originally and also from one another.

External graded differentials—In whole of the nature no cell population grows equally in all directions and thus develops with strict spherical symmetry. This would be only possible if the

external environment had no influence at all on the cells and if any influence present operated continuously and equally on the populations in all direction (a homogeneous environment). But it seems difficult, since such conditions probably do not exist in nature and probably could not even be achieved experimentaly. Moreover, every cell population sooner or later is exposed to the external enviromental differentials almost primitively gradient in nature. In such a gradient, one side of the aggregate would be exposed to a higher, the opposite to a lower concentration of whatever is graded. Differences in the concentration of the graded component on one side in contrast to that on the either upon the "steepness" or slope of gradient and or upon the size (diameter) of the aggregate.

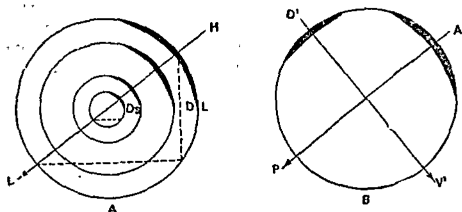


Fig. 182. A—Origin of polarity in an originally symmetrical cellular aggregates ; B—Origin of bilateral symmetry in response of two external environment gradients.

In this way as indicated in the diagram that how cells on opposite sides of an aggregate as it increases in size become exposed to increasingly different microenvironment from Ds (small differences) to DL (large differences). However, when the differences become great enough, cells on the side would respond but not on the other side and thus begin to become different. The originally spherically symetrically aggregate would then be polarized and would exhibit radial symmetry around an axis of polarity passing through the population in the direction of the external gradient.

However a second gradient of sufficient intensity in the external environment could lead to the differential response of the cell located on two opposite sides of the aggregate (Fig. 182 B). The first gradient A—P, might established the anterior posterior axis of

polarity. The second in a plane perpendicular to the first, might establish the dorsal ventral axis. In this way, the aggregate would become bilaterally symmetrical. A very significant point here to be noted is that graded differences in the composition of the environment external to the cell population along with graded differentials in the internal environment of the population constitute a pattern of diverse cellular microenvironment to which the cells may respond by becoming different.

An example in the nature of such a type of hypothetical cellular aggregate is found in the blood island of the chick embryo. In this cellular aggregate which is located in the developing vascular area which surrounds the embryo body proper ; the outer cells become part of the endothelium of capillaries (formed by the fusion of many blood island). Some of the inner cells become red blood cells, others breakdown and contribute to the blood plasma. Another interesting example of the role of the cellular microenvironment in controlling the behaviour of cells is that of the control of the distinction between the central relatively yolk-poor disc-shaped group of the

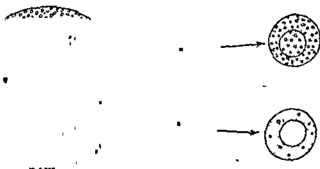


Fig. 183. Influence of the microenvironment on the patterns of intracellular yolk of an early chick blastoderm.

cells in the early chick blastoderm The blastoderm is a disc-shaped population of many thousands of cells located on the top of yolk in a freshly laid but unincubated egg. To this time it can be considered as radially symetrical, although at later stage it does possess a bilaterally symmetrical bias.

Whenever a small groups of central and peripheral area cells are removed (Fig. 183) and grown on the surface of agar culture medium, each group rapidly becomes circular in outline. *Most*

significant point which can be mentioned here is that such an isolated piece, above a minimum size, develop central and peripheral areas, following the radially symmetrical pattern of the whole blastoderm with respect of the location of yolk-poor and yolk-rich cells and in respect of the same proportions of each. It may further be noted here that such isolates remain radially symmetrical, never developing any anterior and posterior polarity and thus no embryo body.

Switch mechanism—The aggregated cells are consuming oxygen and releasing carbon-dioxide. The oxygen tension and carbon dioxide tension will be different on the inside of the aggregate from that what they are at the outer surface. Example of such a decisive role played by the tension of carbon dioxide in controlling the direction of differentiation of cells are found in diverse organisms, such as water molds, slime bacteria, *Hydra*, fishes and birds. This tension has such an impressive role, deserves further study by developmental biologist. Carbon dioxide thus constitutes by its concentrations around a cell a "switch mechanism"—a mechanism which determines which of the two or more capacities of the cells will be used. However, the role of oxygen tension and or humidity as switch mechanisms, has been observed in the differentiation of the cells of the chorioallontoic membrane of the chick extra embryonic membrane system. High oxygen tensions, produced when a "window" in the egg cell is left open direct the cell below the window to synthesize keratin, a product, they normally do not form in detectable quantities. It should be kept in mind here that carbon-dioxide and oxygen are not the only important components of the cellular microenvironment. Other important components would include the myriad of cell products and secretions varying from substances as simple as nitrogenous waste such as ammonia and urea to highly complex protein molecules. Substance such as vitamins, hormones, aminoacids, etc. influence the course of differentiation of certain cells. As the cell respond to their environment, they produce products which are added to their environment. In this way, the cellular aggregate helps to build up and transform its own internal environmental pattern. A cell, thus is not entirely at the mercy of its immediate environment, but as a member of the cell population, a cell is able to contribute to the control of its own differentiation.

The role of the Gene—The problem of interest in biology is

the role of gene which they play in the context of the complex internal environment pattern of a developing embryo. Perhaps the most important single advance in biology during this century has been the indentification of the general chemical nature of the hereditary material of the cell. It is now known, beyond doubt, that the genes, the units of inherited information located on the chromosomes are specific parts of long-chain molecules belonging to the class known as deoxyribonucleic acid (DNA). Mainly, but not exclusively, in the cytoplasm of the cell, another kind of the nucleic acid, ribonucleic acid (RNA), is localized in the ribosomes, the sites of proteins synthesis. RNA like DNA is probably an information-bearing substance. In addition, there is a special kind of RNA called "messenger" RNA, which carries genetic information (presumbly a chemical code based on the order or chemical groups making up the DNA molecule) from the gene (DNA) to the cytoplasmic ribosome where it is used to guide the pattern of protein synthesis—a most fundamental feature of the cell differentiation at the molecular level. *The gene thus acts as a set of instruction*, through messenger RNA and ribosomal RNA the multiplicity of chemical activities at the basis of the behaviour and specificity of a cell.

It is just clear from the result obtained with nuclear clones that the changes which occur in nuclei during development of the frog egg are quite stable. Repeating transplantation of a differentiated nucleus, that is, one incapable of supporting normal development, back into egg cytoplasm is a critical test of its stability. In this way, the nuclear differentiation is not system-independent, the changes which a nucleus has undergone in response to the cytoplasmic environment seems to be quite stable in the absence of their initiating cause. The stability of nuclear clones is particularly significant in that it demonstrates the nuclear differentiative changes are propagated through many cell generations. An interesting suggestion is that propagation of nuclear differntiative changes constitute a special device for stabilizing cellular differentiative changes. This can hardly be questioned in cases of nuclear differentiation involving the orderly loss of chromosomes, such as occur in gall midges and other insects. In the gall midge the germ line cell contains 40 chromosomes, the somatic cell only eight. What role the nucleus plays is less clear, but the fact that the nucleus does participate in governing the protein synthetic

pattern of a cell suggests that it may play an important role in at least temporarily stabilizing the properties of a differentiating cell.

Model of cell differentiation—Cell differentiation is the result of an interplay of the cell's inheritance and environment. A model, designed to pin point this fact, is diagrammatically presented in the (Fig. 184). The model is based on the fairly reasonable assumption

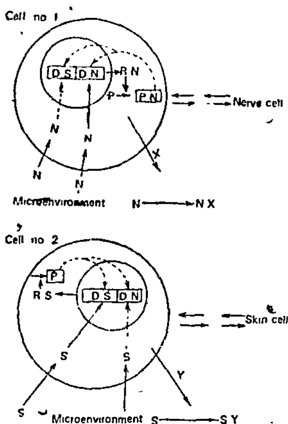


Fig. 184. Models showing how genes activity pattern of a cell is controlled by its microenvironment.

that all the cells derived from a single egg have the same genes. It also assumes, on perhaps less reasonable grounds, that many of the cells of an embryo have inherited cytoplasmic compositions insufficiently diverse to play any directive role in their differentiation. The model consists of two cells, labelled 1 and 2 having initially identical nuclear and cytoplasmic composition. Cell No. 1 is normally in, or experimentally placed in micro-environment type N, whose composition directs its differentiation towards that of a nerve cell. Cell

No. 2 is in a different micro-environment, S, which directs its differentiation towards that of a skin cell. However only two different genes are involved obviously, an extreme simplification of a mechanism probably involving many genes. Also, only one environmental component is considered as the effective one—again, possibly but not necessarily an over simplification of the role of the environment. The two gene activity patterns are probably achieved in the progressive fashion, and, consequently, the distinctive properties of the cell types would be gradually attained. However, fully established patterns of gene activity may vary greatly in the degree of their stability.

The sequence of presumed events leading to the progressive differentiation of cell No. 1 may be summarized as follows. Specific molecule, N, in the environment enter the cell pass through the cytoplasm and then into the nucleus. One or more N, molecules stimulate the specific gene, DN, to direct the synthesis of a correspondingly specific RN molecule (perhaps me senger RNA). Other N molecules may support the activity of the DS gene (which fends to direct the synthesis of keratin, characteristic of skin cells). The RN molecules, bearing a copy of the DN gene code pass out of the nucleus and in the cytoplasm, (presumbly at ribosome sites) direct the synthesis of a specific kind of protein molecule, PN, characteristic of the nerve cell. The PN molecules may interact with other molecules in the cytoplasm, may in some indirect way promote further activation of the DN gene, or may add in repressing the DS gene. In this way, the presence of PN molecule may initiate an endogenous cell stabilizing mechanism. The newly synthesized PN molecule may also bring about a change in the kind and quantities of products, X, which the cell releases into its environment. In this way the cell would begin to control its own environment and thus its further differentiation and stability. However, the same type of mechanism would operate in controlling differential gene activation and suppression in cell No. 2 with the only difference from cell No. 1 that in its micro-environment it contains specific molecule of a type, S, which direct its differentiation into that of the skin cell. Thus the model illustrate well how a single kind of specific molecule in the cellular environment may direct *cell differentiation*. One important feature of intracellular differentiation which is not indicated in the model, that it is mechanism by which the architectural pattern of the cell is controlled by the gene

pattern action. The reason for the omission is very simple, because practically nothing is known about how genes control the synthesis of specific ultra structural cell components in definite localized regions of the cell.

Supracellular pattern of cell differentiation—A striking feature of cellular differentiation in the normal development of multicellular organism is the geometrical pattern in which the various cell types appear in the cell population. The pattern of arrangement of cell types into tissues, tissues into organs and organs into a unified whole organism obviously has developed under the control of certain integrative mechanisms and guidelines. Whatever these guidelines and unifying mechanisms are, it is obvious that they exercise an over all control over every individual cell differentiation process. It is well clear that the nature of cell differentiation is always guided by the guidelines and part of the frame work are inherited and that other guidelines and the parts of the frame work are progressively built up by the developing organism. Now, what is the origin of this framework which is inherited by the developing embryo. There is no doubt about it arising within the egg as it matures in a rather microenvironmen established through activities of the follicle cell which surround the egg in the ovary of the mother organism. Differentials, perhaps gradient in form, are probably present in the microenvironment of every egg, and these could specify polarity and other symmetrical features of the frame work in the maturing egg. In this way inside and outside differentials coupled with the gradient differentials caused for the origin of heterogeneity in a cell population, lead to the formation of a more or less complex cyto-architecture in the egg. Now the extension of the guidelines occurs. However, during the subdivision of the mature fertilized egg by cleavage, its cytoarchitectural pattern or frame work become cut up by the cleavage plants. But this does not in any sense disrupt or change the geometrical feature of the frame work in any significant way. Thus, no matter how the geometry of the cleavage planes is related to that of the egg pattern. The pattern of the egg is transmitted to the cell population derived from it with a minimum of distortion. However, if the tissue movements and differential growth occur even the morphogenetic cell, the pattern in the strict sense is not disrupt or lost but is modified and deformed in an orderly way.

At last a few aspects of differentiation have been described

some details. Undoubtedly, the problem of cell differentiation will in have to be solved through a consensus of imaginative and enquiring minds.

SUMMARY

The differentiation of cell is one of the most interesting phenomena of the living world that occurs during the development of an individuals. The cell differentiation means a cell becoming different. One can distinguish the three major catagories of change, *i. e.* differentiation in time, differentiation in space and differentiation in shape. The differentiation in time is the process through which adult tissues are formed from the cells by becoming different. It is technically known as histogenesis. The differentiation in space however pointed out toward the process by which the egg becomes divided into many distinct different region. It is generally known as regionalization. The differentiation in shape proceed with the change in the shape of cells and known as morphogenesis Thus on the basis of these type of differentiation two aspect of the cell division can be understood, *i. e.* intracellular and intercellular. The intracellular differentiation are found in unicellular organism and also in the cell during reproduction and regeneration. The intercellular differentiation is the process of appearence of different type of cells in a population.

The mechanism of cell differentiation (inter and intracellular)is difficult to understand but we can only examine certain facts about it. There are two general way by which the diverse type of cells are formed. The first is the inheritance of different kind of cytoplasm in the egg. The second is due to response to different microenvironment. It is most probably the cytoplasmic inequality which brought about all the changes the developing cells. Many experiments and observations by different embryologist suggest that during early development, a sorting-out process is at work which segregate into different blastomeres distinct ooplasmic substance which possess different developmental potencies. Moreover, certain experiments have pointed out that nuclei in the early blastomeres are qualitatively equal. The early development and division is *purely a quantitative and depend*

upon the presence of absence of cytoplasmic instruction. Microenvironment also play an important role in the differentiation.

All the experimental approaches pointed out to such problem as the entry of genes into activity in orderly succession as histogenesis, the switching of the developing system into a particular path by a particular type of cytoplasm or by the inductive interaction between neighbouring cells, the co-operation of the cell in the morphogenesis and so on. These are the method by which the biology is feeling its way towards solving this great problem of organisation and differentiation.

At present we are certainly still far from understanding how developing system are actually organised. It seams that it will take the long time to have the clear understanding of it. Perhaps these are three main problem regarding it.

(1) The first is that what may be the nature of change that renders a cell competent, so that it is ready to be switched into a particular developmental path ?

(2) What is the trigger of the switch and puts the cell into the state of determination, which is only with difficulty reversible, and can normally be transmitted through several cell generation ?

(3) At last how are the activities of all the genes concerned in any developmental pathway tied together, so that they proceed in an integrated and orderly manner—or does this, perhaps, follow from the answer of first two questions ?

27

PARTHENOGENESIS

Fertilization is by no means always an essential process in the formation of an individual, even in those animals which normally produce both eggs and sperms. Many animals and plants are known to develop from the eggs without syngamy. This abnormality, the omission of fertilization without the omission of meiosis, has, with a few doubtful exceptions, been observed to give mature progeny only in certain flowering plants of various groups The early stages of development of this kind have been found in some animals and in *Cutleria* by Yamanouchi (1912) where the female gametophyte was protected from fertilization. The egg cell develops without fertilization into the embryo sporophytes. Thus an individual so desired is a parthenot and the process of such development is known as parthenogenesis. (*Parthenos*=virgin ; *genesis*=birth).

Parthenogenesis frequently occurs in lower animals, notably insects, lower crustaceans, and rotifers. In some species, parthenogenesis is the only mode of reproduction ; male individuals being unknown ; while in others a series of parthenogenetic generations is succeeded under the proper environmental conditions by individuals that reduce sexually. In some forms (Hymenoptera) parthenogenesis forms a mode of reproduction and as well as mechanism of sex determination, while in other it is purely a method of reproduction. In parthenogenesis, the chromosomes of the egg are not halved during maturation process, and the offspring, therefore continues with the same number of chromosomes as were present in the parent.

Weissmann (1886-1887) claimed that essential difference between parthenogenetically developing egg and those requiring to be fertilized was that the latter gave off two polar bodies, while the former only one. This is true for a number of types of *thelytoky*, but is by no means a universal rule. Parthenogenesis may be of two kinds.

(1) Artificial parthenogenesis.

(2) Natural parthenogenesis.

(1) **Artificial parthenogenesis**—Artificial means are used tc induce the egg so as to develop parthenogenetically. This process is a recently discovered process, and can be induced in wide variety of animals including annelids, molluscs, echinoderms, amphibians, birds and also in some mammals. Frog egg may be stimulated to develop without fertilization by pricking them slightly with a needle that has been dipped in frog's blood. There are also many chemicals which are used for the treatment of egg for parthenogenesis. Hypertonic sea water, fatty acids, alkaloids, foreign blood, serum and a number of other agencies are the important chemical. But with a very few exceptions, these parthenots (induced by chemicals) reach the stage of metamorphosis of sexual maturity.

(2) **Natural parthenogenesis**—This is the phenomena which occurs in nature without any artificial source or means. By this process bees, wasps, ants, etc. maintain their existence in the nature from one generation to another. It is of two types :—

(A) Arrhenotokous parthenogenesis (Haploid parthenogenesis).

(B) Thelytokous parthenogenesis (Diploid parthenogenesis).

(A) **Haploid parthenogenesis or Arrhenotoky**—In a number of animals, the male arises from unfertilized egg, by a form of parthenogenesis, and are consequently haploid, the female being deploid and arising from fertilized eggs. Such a genetic system is reffered as **haplo-deploid or heploidy parthenogenesis** or **arrhenotoky**. Arrhenotoky is a method of sex determination as well as the form of reproduction. The frequency of the males in the population being determined by the frequency with which unfertilized eggs are laid. It is thus characteristic of groups with haploid males that the sex ratio fluctuates rather widely from species to species and also to some extent with environmental factors, showing no particular tendency to confirm to any fix percentage of males.

Arrhenotoky is only known to have arisen about seven times in the whole history of metazoa (Five times in Insects ; once in Arachnida and once in Rotifers). The most important examples of haploid parthenogenesis in animals include those organisms which show the so called Hymenopteran method of sex determination.

Arrhenotoky in Insects—Hymenopteoa, Homoptera, Coleoptera and Thysanoptera are the four important orders which show the haploid parthenogenesis in Insecta. Hymenoptera, as a whole shows this process from primitive sawflies to the most specialized

parasitic and social wasp. Some of the Hymenopterous insects also show the secondary thelytoky.

The parasitic wasp, *Habrobracon*, is a beautiful illustration of Arrhenotoky. The males are haploid having 10 chromosomes while females are with 20 chromosomes . Although Whiting (1932) was able to produce fertilized eggs experimently. But all the females were definitely diploid and no haploid female could be deducted. The heterozygous or homozygous stage of certain chromosome segments was discovered to control sex determination. Male occurred when these segments were homozygous or hemizygous as in parthenogenetic male, and females were always heterozygotic for these segments.

The inheritance of genes in Hymenoptera has been extensively studied by Whiting (1932). He has shown that as might be expected the characters of a male depend solely on those of his mother, and that he is homozygous or "pure" for characters for which she is heterogyous. A father's gene pass only into his daughter, infact his son can only be considered by his courtesy. Rare exceptions to his behaviour occur, since diploid males have been found.

Moreover in arrhenotoky, eggs develop after undergoing the reduction division. It therefore possesses only half number of chromosomes. Thus the developing male will be haploid. In bees the queen is supposed to determine whether the egg get fertilized or not. If the sperms are given off, when the egg passes down through the oviduct and seminal vesicle, it gets fertilized and the female is produced. If however, an egg slips past the seminal receptacle without being fertilized the result is the male (drone). These male drones are the maters of the future queens and usually supply spermatozoa for the next generation of eggs. Since the males are already haploid they can not undergo reduction division during gamete formation. A sort of vestigial reduction division takes place and with the result of this, two types of cells are formed, one tiny cell without any nucleus and a larger cell with all the chromosomes. A second maturation division divides the chromosomes longitudinally, thus gametes are formed which with the result of syngamy form the female offspring.

Greenshield (1946) suggested that as soon as the hymenopteran type of Arrhenotoky first arose, the male may have been diploid, and the females tetraploid relation. There exist one species in sawfly which exhibit the diploid and tetraploid relation. Schrader

(1920) and Thomsen (1927) indicate that it is not known in white flies (Homoptera) whether all males are haploid. They further pointed out that the haploid males of aleurodids have only a single maturation division which is equational in type in their spermatogenesis.

The occurence of arrhenotoky is very much doubtful in Thysanoptera. Shull (1917) indicated in *Anthotrips verbasi* that virgin females produced all male suggesting a haploid example. Davidson and Beld (1931) also performed certain experiments on the breeding and indicate haploid parthenogenesis in *Franklenlella*, but there is no definite support for that.

In Coleoptera, *Micromalthus debilis* possesses haploid males. The anomolous spermatogenesis occur in male. The vestigeal division takes place and due to this division neither protoplasm nor cytoplasm divide. This is the first division. The second normal division occur (mitotic division) thus producing two sperms from each spermatocyte.

Arrhenotoky in Rotifers—The group Rotifera exhibits numerous variation in their life cycle. In *Bolelloidea* males are unknown and thelytoky is only the mode of reproduction. The certain families of Rotifera also show the complicated alteration of parthenogenetic and sexual reproduction. The male is usually dwarf and usually develop from unfertilized eggs which have undergone two meiotic divisions and they are haploid in constitution.

Arachnida also shows the haploid parthenogenesis. This most appear in ticks and mites. Schrader (1923) has demonstrated male haploidy in 'red spider' and Patau (1936) in *Pediculoides ventriconus*. Opperman (1935) reported male diploidy (Helytoky). Whiting (1945) on the basis of the factors concluded that probably arrhenotoky arose only once in the evolution of the group.

Diploid parthenogenesis or Thelytoky—Here the reduction is abortive and diploid eggs are formed which develop without fertilization. Two main types of thelytoky exists.

(A) Complete Parthenogenesis—Here the species consist exclusively of males or of females with very rare males and every individual arises from an unfertilized egg. Complete parthenogenesis constitute a highly distinct type of genetic system in which sexuality has been entirely abolished and recombination of genes in different individuals is no longer possible (although in some types of complete parthenogenesis segregation of genes present in the same

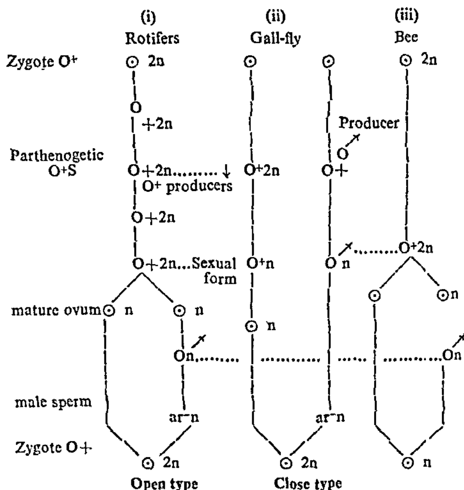


Fig. 185. 3 cycles involving haploid parthenogenesis.

individual). This type of reproduction occurs sporadically in a large number of groups, but never seems to have become widely established in any of the major group of the animal kingdom.

(B) Cyclic parthenogenesis or Heterogony—Here one or more parthenogenetic generations alternate with a bisexual one, usually in an annual cycle, as in the aphids, galls, wasps, rotifers, Cladocera and many parasitic worms.

The various forms of vegetative reproduction ('budding') which exist in such groups as the corals, Bryozoa and tunicates may be genetically similar to parthenogenetic reproduction.

In the aphids, there is a succession of females reproducing by diploid parthenogenetic eggs before the sexual forms are again produced. In *Phylloxeran* only two such forms occur before the sexual forms are produced.

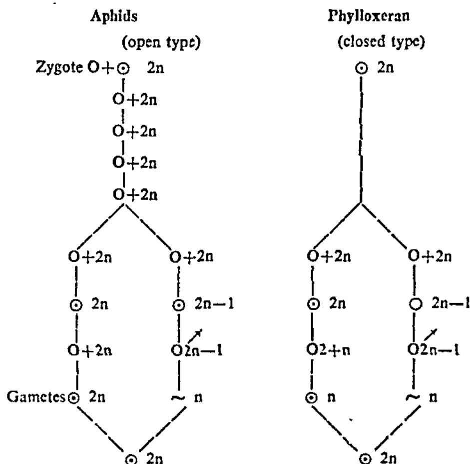


Fig. 186. Cyclic parthenogenesis.

Cytological details of Thelytoky—From the cytogenetic point of view we may draw fundamental distinction between two major types of thelytoky :—

(A) Meiotic type—In this process meiosis takes place in the

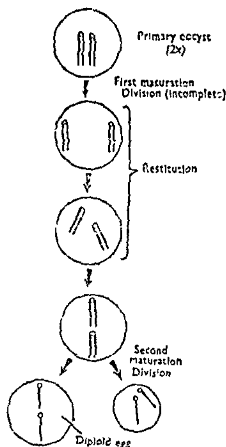


Fig. 187. *Origin of thelytoky by restitution.*

egg, and the chromosomal reduction occurs which is 'compensated' for by the doubling of chromosomes number at some stage in the life cycle. Suomalainen (1950) called it as automatic parthenogenesis. The doubling of chromosome occurs by two processes : (i) By restitution and (ii) By auto fertilization.

1. By Restitution—In this method chromosomes pair, split and become separated to the opposite poles of cell. But cell itself does not divide into two as usually happens. The chromosomes first come together and then in the middle of the cell. This courting chromosomes together is called *restitution*. Now the eggs and polar body is formed by the second meiotic division. In the right sense the first meiotic division is completely omitted thus giving the doubling number of chromosomes. This process generally occurs in many lepidopteran insects

2. By auto-fertilization—The mutual fusion of polar body nucleus and the egg nucleus is called auto-fertilization. By this process diplosis occurs.

Meiotic parthenogenesis—It occurs in a large number of animals of various groups.

1. Hymenopteran type—Thelytoky also occurs in Hymenoptera. It is supposed that thelytoky have arisen from arrhenotoky through the process of modification in the maturation divisions in the egg so as to produce diploid egg. In parasitic wasp, *Nemeritis conesceus* meiotic thelytoky occurs and the first maturation division is incomplote and the chromosome separated come again together by the process of restitution.

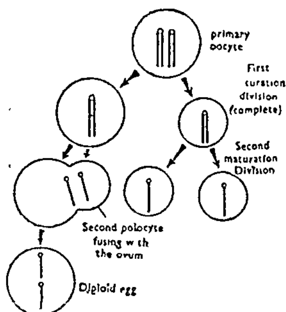


Fig. 183. Origin of thelytoky by auto-fertilization.

1. Lepidopteran type—Seiler (1948), Narbel (1950) and Pardi (1950) have studied several family of the order Lepidoptera in which the meiotic parthenogenesis occurs. These are the different ways of meiotic thelytoky in different animals. In *Solenobia triquetrella* three 'races' are known: a diplold bisexual one, a diploid thelytokous, and a tetraploid parthenogenetic race. The eggs of the parthenogenetic females (diploid and tetraploid) undergo a true meiosis and thus form the two polar bodies. The further, mechanism is not normal and the first and the second meiotic cleavage division take place with the reduced chromosome number but after second meiotic cleavage, four nuclei fuse in pair to restore original somatic number.

3. Artemia salina—In this animal usual first meiotic division occurs but no second division. Thus only a single body is formed. In this process the diploid number is restored by the fusion of products of second meiotic division.

(B) Ameiotic Type—In this process the meiosis has been entirely supressed, the maturation division or divisions in the egg being mitotic in nature. It generally occurs in a number of arthropods (crustacean and insects).

1. Mortimer (1936) has pointed out ameiotic parthenogenesis

in *Dephnia pulex*. Mattox (1937) pointed out the *Compelona refum* (a fresh water mollusc) reproduce by ameiotic the thelytoky. Certain Isopoda (*Trichoniscus*) shows the polyploid parthenogenesis. In this cyclical or heterogony type of thelytoky occurs.The diploid *Trichoniscus* always occurs in equal numbers (male and female). The triploid females are ameiotic, their chromosomes do not pair during oogenesis and only one maturation division occurs which is mitotic in nature. The unfertilized eggs, which develop into the adult possess the somatic number of chromosomes.

In insecta, long horned grasshopper and wingless tettigoniid, no male occurs and reproduction is purely brought about by the process of ameiotic thelytokus. Matteby (1941) pointed out that in this process no pairing of the chromosomes occurs and only one polar body is formed. *Thus diploid eggs are produced.*

Suomalainen studied about seventeen parthenogenetic species of weevils. In this only one equational maturation division occurs in the egg. Thus diploid eggs are produced, which develop parthenogenetically.

Thus it can be concluded although almost no genetic work has been carried out on parthenogenetic higher organisms, it is interesting to consider the genetical principles which should apply in such cases. We should accept the genetic consequences of ameiotic and meiotic thelytoky to be quite different. In ameiotic parthenogenesis genetic segregation will not occur. Recessive mutations and structural rearrangements will try to accumulate indefinitely in such organisms, only the one which are immediately deliterious being eliminated by natural selection. Such forms must consequently be expected to become gradually more and more heterozygous.An ameiotic form evolving for a long period of time, might be expected eventually to lose its diploid characters in both the cytological and genetical sense, its two *chromosomes sets having become almost unlike. Moreover,* since no pairing of chromosomes takes place during the maturation of the eggs, there is no mechanical barrier to the establishment of any type of polyploidy, in such forms and various forms of aneuploidy, due to irregular reduplication of some chromosome elements, must be expected to occur.

In meiotic parthenogenesis segregation may occur in the offspring of a single female, if she is heterozygous. Hetrozygosity, will, however, be very rare in such forms, irrespective of the stage at which the doubling of the chromosome number occurs, since in the

absence of sexual reproduction, it only arises by mutation and will be eliminated or greatly reduced at each successive meiosis. Thus all organisms with meiotic thelytoky will tend in practice to be homozygous for almost all loci ; in this respect they possess a genetic system which is the antithesis of that found in species with a meiotic thelytoky.

Special types of Parthenogenesis :

1. **Gynogenesis**—A rather peculiar type of parthenogenesis which has been called gynogenesis or pseudogamy or pseudofertilization occurs in a number of species of nematodes, planarians, and earthworms. In these forms the egg has to be stimulated to develop by the penetration of the sperm, but the later then degenerates and no true fertilization occurs (in the genetic sense). Gynogenesis seems to be a frequent type of parthenogenesis in the groups where the sexually reproducing forms are hermephrodite. An analogous phenomena also occurs in some plants.

Alloecoel turbellarians also provides a unique example of a typical parthenogenesis. In *Bothrioplana semperi* egg capsules contain two nuclei both of which contribute to the developing embryo. Just before the first maturation division a peculiar process of chiomosome "doubling" occurs followed by the reduction division at the second division.

Apospory may in its genetic effects,be considered as a variety of diploid parthenogenesis. It occurs in flowering plants and also in certain forms. When it occurs it also gives rise to diploid offspring by the development of two fused embryo sac cell. All these forms of diploid parthenogenesis gave the same genetic results.

Singnificance of Parthenogenesis.

1. **As a method of Reproduction**—Some of the immediate advantages of parthenogensis as a method of reproduction are fairly obvious. This is the simple process of maintaining the existance of individuals.By dispensing with the need of mating it allows the whole of adult life to be devoted to feeding and reproduction. The production of parthenogenetic forms is always higher than that of the related bisexul forms.

2. **As a device for high multiplication**—Certain insects (aphids) show the cyclical parthenogenesis. They exhibit alternation of generations and multiply very rapidly in the parthenogenetic cycle. During the sexual generation recombination of genes can take place.

3. **Elimination of non-adaptive characters**—As regards the genetic control, the parthenogenesis is not suitable for the organism. Natural selection in the parthenogenetic forms consist in the elimination of the non-adaptive combination of genes.

4. **Escape from sterility**—Darlington (1939) pointed out the parthenogenesis as an 'escape from sterility'. The sterility might be due to triploidy, pentaploidy or to incompatibility of gametes arising from genetic cause.

There is every reason to blieve that in the diploid type, the parthenogenetic mode of development has been derived from the normal sexual cycle, involving a supression of meiosis and in monoploid type by an adjustment of development to a different nuclear constitution. The supression of meiosis is suggested by the various observed conditions intermediate between meiosis and ameiosis, and between facultative and obligatory parthenogenesis and also by the fact that in certain plants parthenogenesis occurs only after a failure of meiosis. Since the monoploid parthenotes occasionally encountered among plants and animal species do not develop so well as the diploid zygotes, it may be suggested that the ability to undergo arrhenotoky development with a single genome may have been slowly acquired. Moreover, when the parthenogenetic development of monoploid eggs is induced by artifical means, the few individuals that are successful in developing through metamorphosis have almost invariably become diploid. In the mitosis occuring in parthenogenetic embryo of frog, for instance, both the monoploid and diploid numbers are found, indicating a gradual doubling process. Evidently the embryos not undergoing this change fail to survive.

Development in the monoploid condition is therefore a possibility in animals as it is in plants, provided the condition favouring it are present. Monoploid animals, as a rule, derived by such means are almost sterile. Among natural parthenotes, however, fertility may obtan. All these facts indicate, that in the evolution of various groups of organism, there have been a adjustment of reproductive process to a considerable ranges of variations in the nuclear cycle. This is further evident in plants, most of which have reproductive cycles differing widely from those in animals.

SUMMARY

The egg cell develops without fertilization into the embryo sporophytes. The individual so formed is a parthenot and the process of such development is known

as parthenogenesis. This process frequently occurs in lower animals, (such as in insect, lower crustacean, and rotifer) and plants. In some species, parthenogenesis is the only mode of reproduction. The parthenogenesis may be of two kinds (1) Artificial parthenogenesis and (2) Natural parthenogenesis. In the artificial parthenogenesis, some means is used to induce the egg so as to develop, while natural parthenogenesis is the phenomena which occurs in the nature. The natural parthenogenesis may be of two types.

(a) Arrhenotokus parthenogenesis or Haploid type, in which the male arise from unfertilized egg. This occurs mainly in Hymenoptera, Homoptera, Coleoptera and Thysanoptera. Hymenoptera, as a whole shows this process from primitive sawflies to the most specialized parasitic and social wasp. Arrhenotoky in rotifer exhibits numerous variation in their life cycle. Arachnida also shows the haploid parthenogenesis.

(b) **Thelytoky or Diploid parthenogenesis,** in this the reduction is abortive and diploid eggs are formed which develop without fertilization. Two main type of thelytoky exists, (a) Complete parthenogenesis, in which every individuals (male or female) arises from an unfertilized egg and (b) Cycleic or Heterogony parthenogenesis, in which one or more parthenogenetic generations alternate with a bisexual one, usually in an annual cycle, as in the aphids, galls, wasps, rotifers, cladocera and many parasitic worms.

The parthenogenesis is a very important phenomenon and is a very much significant in the life of lower animals and plants. It provides a method of reproduction, device for high multiplication, elimination of non-adaptive characters and escape from sterility.

GLOSSARY

Acentric—A part or the whole of a chromosome which lacks the centromere.

Allelomorph—One or two dissimilar genes which on account of their corresponding position in corresponding chromosomes are subject to Mendelian inheritance in a diploid.

Alternation of generation—The occurrance of the two series of nuclear divisions, *i. e.* haploid and a diploid in the life cycle.

Ameiosis—The occurance of one division of the nucleus instead of the two of a normal meiosis, giving non reduction of the mother cell.

Amitosis—Direct nuclear division, without the separation of daughter chromosomes.

Amphimixis—To bring together the elements from two gametes in fertilization (*opposed* to apomixis).

Anaphase—A stage at which daughter chromosomes move apart in the nuclear division.

Androgenesis—It is the parthenogenesis by male.

Aneuploid—Having the different chromosomes of a set present in different numbers, therefore an unbalanced polyploid.

Animal pole—The point of the surface of an egg at which the polar bodies are formed ; one end of the egg-axis (*opp.* vegetal pole).

Anisogamy—The fusion of gametes of unequal sizes.

Apogamy—Apomixis involving the replacement of the gametes by unspecialised cells which do not fuse.

Apomixis—The occurrance of the external form of sexual reproduction with the omission of fertilization and usually meiosis as well.

Apospory—Apomixis involving the replacement of the spores by unspecialised cells which have not undergone meiosis.

Asexual reproduction—Reproduction without the formation and union of gametes (*opp.* sexual reproduction).

Asynapsis—The non pairing of chromosomes at meiosis.

Attachment—(i)The position of the centromere. (ii) The permanent fusion of two chromosomes.

Autosomes—The group of chromosomes whose segregation do not normally affect the determination of sex ; any chromosome other than the sex chromosome.

Azygote—Organism arising from haploid parthenogenesis.

Basic number—The supposed number of chromosomes found in the gamete of a diploid species of a polyploid series.

Biogenesis—The production of living things from living things, not from non-living things (opp. abiogenesis)

Bivalent—The two homologous chromosomes paired at meiotic metaphase.

Cell—A unit in the structure and function in animals and plants, a mass of protoplasm containing one or several nuclei.

Cell division—The method of origin of new cells from pre-existing ones.

Centromere—A particle in the chromosome filament whose special cycles of repulsion and division determine the anaphase and terminalization movements of the chromosomes.

Centrosomes—The self propagating body which during division in many organisms lie at the two poles of the spindle and appears to determine its orientation.

Chiasmata—An exchange of partners in a system of paired chromatids ; observed between the diplotene and the beginning of the first metaphase in meiosis.

Chiasma theory of pairing—A hypothesis, that whenever two chromosomes which have been paired at pachytene remain associated until metaphase ; they do so by virtue of the formation of a chiasmata or visible exchange of partner amongst their chromatids.

Chiasma type theory—(i) The theory that chiasma are connected with crossing-over either as a cause or as a consequence. (ii) It is special assumption that chiasmata are determined by crossing-over between two non-sister chromatids of the four involved.

Chondriosomes—Self propagating bodies in the cytoplasm such as mitochondria, and Golgi bodies.

Chromatid—A half chromosome between early prophase and metaphase of meiosis and mitosis.

Chromatin—The part of the chromosome that stains deeply during cell division, consisting mostly DNA (*opposed* to **achromatic**).

Chromocentre—Fused prochromosome or body produced by fusion of the centromere.

Chromomeres—The smallest particle identifiable by its characteristic, size and position in the chromosome thread between leptotene and pachytene and in salivary gland nuclei.

Chromonemata—The chromosome thread at leptotene and pachytene.

Chromosome—One of the bodies into which the nucleus resolves itself at the beginning of cell division and from which it is derived at the end of the cell division : composed of a matrix containing at least two chromonemata.

Chromosomal aberration—Any loss or gain of a part of a particular chromosome, of a whole chromosome or of a haploid set of chromosomes ; may give rise to a heritable variation.

Chromosome thread—The thread consisting of centromere, chromomeres, achromatic connective thread and perhaps pellicle at prophase ; and constituting as a spiral, the metaphase chromosome.

Coil—Spiral, a coil of the chromosomes thread at mitosis or meiosis.

Complement—The group of chromosomes derived from a particular nucleus in gamete or zygote, composed of one, two or more sets.

Condensation or **contraction**—The thickening of the chromosomes or shortening and spiralisation of the chromatids during prophase.

Configuration—An association of chromosomes at meiosis.

Congression—The movement of chromosomes on to the metaphase plate.

Conjugation—The pairing of chromosomes, gametes or zygote, or the fusion of pair of nuclei.

Constr.ction—An unspecialised segment in the metaphase.

Crossing-over—The exchange of corresponding segment between corresponding chromatids of different chromosomes by breakage and reunion following pairing.

Cytokinesis—The separation of daughter cells, usually after nuclear division.

Cytology—The science of the structure of cells.

Cytoplasm—The protoplasm apart from the nucleus.

Daughter chromosomes—Chromatids.

Diakinesis—The last stage in the prophase of meiosis.

Diploid—Having two sets of chromosomes forming homologous pairs, as in somatic cells primordial germ cells and zygote (2n). opp. haploid.

Diplophase—(i) The diplotene stage of meiosis (ii) The diploid phase of the life cycle.

Diplotene—The stage of meiosis which follows division of the chromosomes at the pachytene stage.

Disjunction—The separation of chromosomes of a homologous pair at anaphase.

DNA—*A nucleic acid found mainly in chromosomes considered to* be a primary substance of the gene.

Duplication—The occurrance of the segment twice in the same chromosome or in the same complement.

Dyad—(i) The pair of cells formed after meiosis. (ii) The univalent chromosomes, composed of two chromatids, at meiosis.

Embryo sac—The female gametophyte in angiosperm.

Factors—See genes.

Fertilization—The fusion of the two germ-cells and of their nuclei, to form a single cell, the zygote.

First division—The first of the two meiotic division formerly known as the "**heterotypic**" or '**reduction divison,**"

Gametes—Germ cells which are specialized for fertilization and can not normally develop without it.

Gametogenesis—The differentiation of gametes.

Gene—*The unit of reproduction and hence of crossing-over in the* hereditary material.

Genetic—A property possessed by an organism by virtue of heredity.

Genome—A set of chromosomes (n) inheretid as a unit.

Genotype—The kind or type of hereditary properties of an individual organism.

Golgi apparatus—Bodies in the cytoplasm, so called in anticipation of their proving to an apparatus.

Haploid—Having a single set of chromosomes which do not occur in pairs, as in gamete (n). *Opp.* Diploid.

Heredity—The process by which like begets like (in sexual reproduction.)

Heterochromosomes—Chromosomes with exceptional form or behaviour, especially at meiosis, such as sex chromosomes fragments.

Heterogametic—Producing gametes of two kinds with regards to their properties of sex determination.

Heterogamy—Differenciation of male and female types of gametes.

Heterozygote—A zygote *derived from the union of gametes, dissimilar* in respect of their chromosomes or from mutation in a homozygote.

Homology—The similarity of structures in different organisms, which they owe to the common *ancestry of the organisms.*

Homologous chromosomes—A pair of chromosomes similiar in size and shape, one of which is contributed by each of the gametes, that unite to form zygote.

Homozygote—A zygote derived from the union of identical gametes in the number of chromosomes.

Hybrid—Heterozygote.

Interchange—An exchange of non-homologous terminal segments of chromosomes

Interkinesis—*Interphase, resting stage.*

Inversion—The reversal of the linear sequence of the genes in one segment of a chromosome relative to the centromere.

Isogametes—Gametes of equal size that unite in pairs.

Isogamy—Fusion of gametes of equal size.

Karyokinesis—Mitosis.

Karyology—Nuclear cytology.

Karyomere—A compartment or vesicle in the resting nucleus usually containing one chromosome.

Leptotene—The single chromosome's *thread at the early stage of* prophase of meiosis.

Matrix—A space left round the solid part of a metaphase or anaphase chromosome when it contracts.

Maturation—The formation of gametes or spores by meiosis.

Meiosis—A form of mitosis in which nucleus divides twice and the chromosomes once, cells arising after meiosis contain the haploid number of chromosomes.

Metaphase—The stage of mitosis or meiosis in which the chromosomes *lie in a plane at right angles to the axis of the spindle* and half way between the poles.

Mitosis—The process by which the daughter chromosomes are separated *into two indentical groups longitudinally, diagnostic* property of division of the nucleus : cells arising after mitosis contain the diploid number of chromosomes.

Mother—The cell with a diploid nucleus which by meiosis give rise to four haploid nuclei.

Non-disjunction—Cytologically, the failure of separation of paired chromosomes at meiosis and their passage to the same pole.

Non-homologous pairing—*Association of non homologous parts* of chromosomes at pachytene.

Nuclear sap—The fluid which is lost by the chromosomes as they contract,—during prophase and which fills the space of nucleus.

Nucleolus—*A body in the nucleus which disappears and does not* resolve itself into chromosomes at mitosis. Rich in RNA.

Nucleus—The part of the cell containing, the chromatin and limited by the nuclear membrane ; a cell body reproducing by mitosis.

Oocyte—Egg mother cell.

Oogenesis—The differentiation of the ova.

Orientation—The movement of chromosomes so that their centromeres lie axially with respect to the spindle.

Pachytene—The double thread (and the stage at which it occurs) produced by pairing of the chromosomes in the prophase of meiosis.

Pairing of chromosomes—(active) The coming together of chromosomes at zygote or (passive) the continuance of their association at the first metaphase of meiosis.

Parthenogenesis—A form of apomixis in which the female gamete *develops without fertilization.*

Phenotype—The external appearance produced by the reaction of organism of a given genotype with a given environment.

Plasmagene—A unit in the cytoplasm causing hereditary trait, *e. g. Kappa* in Paramecium.

Polar bodies—The expelled products of the division of the occyte nucleus in animals at the time of oogenesis.

Polarisation—Of chromosomes at telophase of mitosis and later the maintenance of their proximal parts on the polar sides of the nucleus.

Pro chromosome—Condensed proximal part of a chromosome.

Prometaphase—Stage between the dissolution of the nuclear membrane and the congression of the chromosomes on the metaphase plate.

Prophase—The stage in mitosis and meiosis from the appearance of the chromosomes to metaphase.

Protoplasm—The complex colloidal material of which all organisms are composed ; the physico-chemical basis of life.

Protoplast—The protoplasm of one cell.

Reduction—The halving of the chromosome number at meiosis.

Reductional Division—A separation of homologous parts of the chromosomes derived from the opposite parents at anaphase of a first or second division.

RNA—A nucleic acid found mainly in cytoplasm and nucleus. Thought to be material which translate the genetic information of DNA into action.

Set of chromosomes—A minimum complement of chromosomes derived from the gametic complement of a supposed ancestor.

Sex-chromosomes—Chromosomes that differ in number and distribution in the male and females of a species ; the X-and Y-chromosomes.

Sexual differentiation—The production by an organism of gametes of two sizes so that the larger can be fused with smaller.

Sexual reproduction—That which involves meiosis and fertilization.

Sperm (Spermatozoon)—The male gamete in animals.

Spermatogenesis—Differentiation of the spermatozoa.

Spermatocyte—Sperm mother cell.

Spermiogenesis—The cytosomal differentiation of a sperm ; the transformation of a spermatid into a sperm.

Spindle—The axially differentiated part of the cytoplasm within which the centromere of the chromosomes are held during metaphase and anaphase.

Spiral—A coil of the chromosome thread (chromosome or chromatids) at mitosis or meiosis.

(i) *Internal*—a coil within a single chromatid between prophase and anaphase.

(ii) *Relation*—coiling of the two chromatids or chromosomes round one another.

(iii) *Major*—the large internal coil at meiosis

(iv) *Minor*—the smaller internal coil.

(*v*) *Relic*—the coiling which survives at telophase and prophase.

(*iv*) *Super*—larger coils derived in prophase from the rearrangement of relic spirals.

Synapsis—Temporary pairing of chromosomes at zygotene.

Syngamy—Fusion of gametes.

Telophase—The last stage of mitosis after movement of chromosomes has ceased.

Terminalization—Expansion of the association of the two pairs of chromatids on one side of the chiasma at the expense of that on to other side. The socalled because the resulting "movement" of the chiasma is usually if not always towards the end of chromosomes.

Tetrad—Quartet of cells formed by meiosis in a mother cell or the four chromatids making up a bivalent at meiosis.

Tetraploid—An organism having four sets of chromosomes.

Triploid—An organism having three sets of chromosomes.

Univalent—A body at the first meiotic division corresponding with a single chromosome in the complements: especially when unpaired.

W-chromosome—Sometimes used for the **X**-chromosomes where the female is heterozygote sex, **Z** being then used for **Y**.

X-chromosome—With diploid sex differentiation, the sex chromosome in regard to which one sex is homozygous; this is said to be homozygous sex with sex differentiation in the haploid. the sex chromosome of the female.

Y-chromosome—The sex chromosome that is present and pair with the **X** in the sex heterozygote.

Zygote—Cell formed by the union of gametes and the individual derived from it.

Zygotene—The pairing threads (and the stage at which they occur) in the prophase of meiosis.